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Contents

The Salt Requirements of Lupinus albus. C. H. Arndt	1
Effects of Various Methods of Applying Fertilizers on Crops and on Certain Soil Conditions. Dana G. Coe	7
Potassium Ferrocyanide and Ferric Ferrocyanide as Sources of Iron for Plants. C. G. Deuber	23
The Viability of the Nodule Bacteria of Legumes Outside of the Plant: I, II. MARCOS M. ALICANTE	27
A New Soil Core Sampler. E. B. POWELL	53
The Availability of Nitrogen in Garbage Tankage and in Urea in Comparison with Standard Materials. A. L. Prince and H. W. Winson	59
Some Residual Effects of Neutral Salt Treatments on the Soil Reaction. C. H. Spurway and R. H. Austin	71
The Utilization of Water by Plants under Field and Greenhouse Conditions. N. M. TULAIKOV.	75

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THE SALT REQUIREMENTS OF LUPINUS ALBUS

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University of Pennsylvania

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The seedlings of *Lupinus albus* have been found to be so well adapted to certain kinds of physiological work that it became desirable to determine the composition of a solution which would perform the same function in the study of its physiology that Shive's (2) optimum solution has for wheat.

Solution and sand cultures were used and manipulated in general as recommended by the Committee on the Salt Requirements of Plants (1), except for certain details here noted. The solution cultures were renewed every four days. The plants were grown in 500-cc. beakers (tall form) containing 400 cc. of the solution. For the sand cultures 1250 gm. of thoroughly washed "Juniata" sand was placed in liter pyrex beakers (tall form). The water content was held at 62.5 per cent of the water-holding capacity. The salt combinations used were: Series I-K2SO4, CaH4(PO4)2, Mg(NO3)2; Series II -KNO₃, CaSO₄, MgH₄(PO₄)₂; Series III-KH₂PO₄, Ca(NO₃)₂; MgSO₄. In each series three total salt concentrations in respect to the cation were used: X, 0.0021 N; 4X, 0.0084 N; 16X, 0.0336 N. The concentration of the anions was the same except as modified by the nature of the phosphate salt which gave the PO4 ion a normality three times that of the other anions. This particular method of making up the solutions was chosen to facilitate the comparison of chemical equivalents when represented diagrammatically by the triangle system. (For the salt ratios see table 1.) Iron was supplied as ferric citrate in amounts proportional to the total salt concentration of the solutions. All seeds were carefully selected by weight, germinated in moist sand, and the most vigorous 25 per cent of the seedlings was selected for the cultures.

Series 1 was grown in a greenhouse for 25 days during March. The average daily loss from a white spherical atmometer was 15.8 cc. The average daily range in temperature was 24.4° to 18.3°C., with a daily mean of 21°C., and maximum and minimum temperatures of 30°C. and 10°C. Series II was grown in the same greenhouse for 25 days in May. The average daily loss from a white spherical atmometer was 18.1 cc. The average daily range in temperature was from 28° to 14°C. The maximum temperature was 32°C. which was approximated on several days. The latter temperature is probably too high for the best growth of *Lupinus albus*. Series III was grown for 32 days during July and August on an outside lattice work bench which could be covered during rains. The plants were shaded with a translucent white

oilcloth, from 11 a.m. to 3 p.m., on clear days when the temperature exceeded 20°C. The average daily loss from a black spherical atmometer was 24.7 cc. The average daily range in temperature was between 31.5° and 18°C. A maximum of 38°C. was approximated on 3 days. Regardless of the high temperatures prevailing during this experiment, the plants were superior to those grown in the previous series. The condition of the plants in the best cultures indicated that they might have been kept growing indefinitely.

EXPERIMENTAL RESULTS

In table 1 are shown the salt ratios as well as the relative growth in the various solutions. The results are shown graphically in figure 1. The highest yielding cultures as well as the highest average yield for all cultures of a series tend strongly to occur in the 4X concentration. There is an ap-

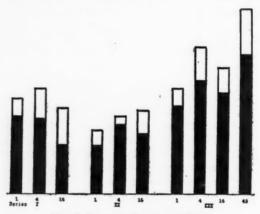


Fig. 1. Relative Growth of Tops

All weights are calculated on the basis of the green weight of culture 9, 4X concentration of series III, as 100 per cent. Solid portion indicates the average yield of all cultures of a concentration; open portion, the relative yield of the best culture; "1," "4," and "16" indicate the total salt concentrations; "S" indicates sand culture.

parent exception to this in series II, 16X. The difference, however, is slight and the high yielding cultures in the high concentration are all relatively low in phosphate.

The roots were generally best developed and of the best color, in the 4X solutions. In the lower concentration they tended to become brownish and soft. The tap roots were short and thick and developed few secondary roots. The secondary roots were best developed in the 16X concentration, except when their development was retarded by the acidity of the solution. The relative average yields of all cultures of the same concentration of a series as well as the relative weights of the roots in the culture producing the highest yield, are shown in figure 2.

The effect of the various salt concentrations on the water requirement per gram of green weight of tops produced is shown in figure 3. The concentration of the solution had relatively little effect on the transpiration per unit weight produced, although there is an indication of a slightly higher

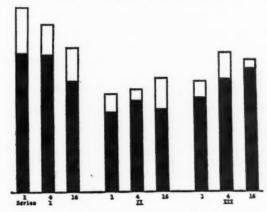


Fig. 2. Relative Growth of Roots, Based on the Dry Weight of Culture 9.
4X Concentration Series III

Labels as in figure 1

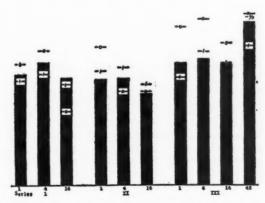


Fig. 3. Relative Transpiration per Gram of Green Weight Produced

Height of the solid portion indicates the average for all cultures; -1- indicates the transpiration for the best culture, -0-, for the poorest; -0- for the sand culture is only $\frac{2}{3}$ of its relative height. Labels as in figure 1.

requirement in the 4X concentration. In most cases, the low yielding cultures show a high water requirement. No conclusion can be drawn concerning the effect of the various salt combinations on transpiration because of

the different environmental conditions prevailing during the growth of each series.

A careful record was kept of the H-ion concentration of the original solutions and of the effect of the growth of the plant on its reaction. The H-ion concentrations of the original solutions ranged in series I, from pH 3.2 to 4.2; in Series II, from pH 3.4 to 4.4; in series III, from pH 3.8 to 4.8. All solutions with an original acidity greater than pH 3.6 gave low yields, even when the reaction of the original solution was quickly changed by the plants. The strong buffer action of the 16X solutions in series I and II probably accounts

TABLE 1
Relative yield of cultures based on the green weight

TUM-		CENTR.			SERTES I			SERIES I	ı		SERIES II	11	SAND CUL-
BER	K.	Ca.	Mg.	x	4X	16X	X	4X	16X	x	4X	16X	TURES
1	1	1	4	123	133	88	72	73	39	59	88	89	74
2	1	2	3	112	122	55	86	90	64	70	108	98	73
3	1	3	2	110	56	35	78	107	77	53	87	94	82
4	1	4	1	107	50	22	43	105	116	71	99	83	74
5	11	11	3	113	100	98	70	87	77	54	71	99	1
6	11	21	21	100	118	54	68	91	62	79	101	90	
7	11	3	11	112	82	38	92	110	78	75	95	99	
8	2	1	3	104	135	84	59	96	37	86	86	79	72
9	2	2	2	108	100	60	81	100	80	79	100	98	100
					14.8†			12†			21.9†		27 .4†
10	2	3	1	93	58	40	71	110	114	76	103	72	75
11	21	11	21	119	134	74	68	106	93	74	63	109	
12	21	21	13	104	88	58	88	110	96	70	114	69	
13	3	1	2	98	95	112	61	104	73	65	94	85	57
14	3	11	11	88	98	94	61	84	95	79	82	79	
15	3	2	1	107	80	53	63	112	96	91	108	98	67
16	4	1	1	97	109	45	46	111	100	59	107	88	40
A	vera	ge		99.7	96.2	63.1	69.6	99.7	88.1	70.6	97.7	86.5	75.
*			ſ	+23	+39	+49	+23	+12	+28	+21	+11	+23	+24
V	anai	tion .		±12	-46	-41	-26	-27	-41	-17	-34	-17	-35

^{*} Same salt combination as series III, 4X concentration. The relative weights of 9 in the X and 16X concentrations were 63 and 82 per cent respectively. The plants in the latter culture were stongly chlorotic.

for the extremely poor yields of the cultures high in phosphate. This was plainly evident when the acidity was associated with a high calcium concentration. The roots were unable to penetrate the solution in cultures 3, 4, 7, and 10 of series I and 16X concentration. The growth of the plants in series II changed the reaction of the solutions only slightly. Certain cultures, peculiarly, tended to become more acid with the limit at pH 3.7. This was the reaction after 4 days in several of the highest yielding cultures. The critical H-ion concentration for Lupinus albus is somewhere between pH 3.4

[†] Weight of no. 9, 4X, in grams.

and 3.6 when the acidity is due to phosphoric acid formed by the dissociation of a monobasic phosphate.

DISCUSSION

A study of the results does not indicate that a certain specific ratio of the cations is required. There is no evidence to justify the term "calciphobe" as formerly applied by ecologists to *Lupinus albus*. When the results are plotted graphically by means of the triangle system, and the anions rather than the cations are placed at the apices, specific effects are shown for certain concentrations of the anions. In the X and 4X concentrations, the concentration of the nitrate ion seems to be a limiting factor. At least, there is a strong tendency for the low yields to be associated with a low nitrate concentration (fig. 4 and 5). As the total concentration of the solution is increased,

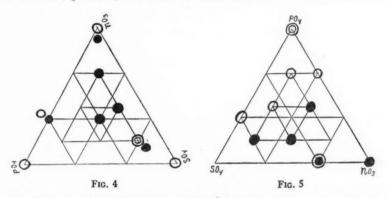


Fig. 4. Graph to Show Distribution of Highs and Lows in Relation to the Anions in the $0.0021\ N$ Salt Concentration

Two highs and lows are taken from each series. The solid circles indicate highs, the open circles lows. Two concentric circles indicate two lows at that point, a solid center indicates two highs.

Fig. 5. As in Figure 4, Except for a Total Salt Concentration of 0.0084 N

the high yielding cultures tend to shift from the nitrate to the sulfate apex, with strong tendencies away from the phosphate apex. This also applies to series III in which acidity was not a limiting factor (fig. 4, 5 and 6). This tendency was particularly well shown in the sand cultures (fig. 7) in which after the beginning of the growth of the plants, a H-ion concentration of pH 5 to 5.6 was maintained. All cultures at the phosphate apex were strongly chlorotic and did not develop normal coloration upon the addition of increased amounts of iron. This was also true of sand culture 9, 16X concentration. The effect of the anions on the yield is clearly shown by a comparison of the average yield of the three cultures at each apex. When the central culture is given as 100 per cent, the yields were: PO₄ apex, 54 per cent;

SO₄ apex, 78 per cent; NO₃ apex, 82 per cent. It must not be forgotten in considering these results that the *normal* concentration of the phosphate ion is three times that of the sulfate and nitrate ions.

The investigations here reported (because of the inherent difficulties encountered in work of this nature) are necessarily only preliminary and any future researches will surely modify the results. The author does feel, however, that he has obtained sufficient information to justify the original object of the investigation. The use of *Lupinus albus* in salt balance work will probably not be continued here because climatic conditions in eastern Pennsylvania are unfavorable for growing it to maturity.

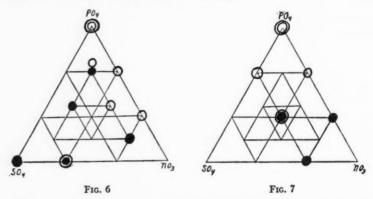


Fig. 6. As in Figure 4, Except for a Total Salt Concentration of 0.0336 N

Fig. 7. Highs and Lows in the Sand Cultures

Open circles indicate lows; open concentric circles, the poorest culture; solid circles, highs; solid center with a concentric circle, the best culture. Total salt concentration, 0.0084 N.

SUMMARY

A very favorable total salt concentration for the growth of *Lupinus albus* is 0.0084 N when calculated in respect to the cations.

The salt ratios should be 5:3:4 for potassium, calcium, and magnesium; or 5:9:4 for the nitrate, phosphate, and sulfate ions, respectively. A solution composed of 0.0035 N KNO₃, 0.0021 N CaH₄(PO₄)₂, and 0.0028 N MgSO₄, will produce a favorable growth when the H-ion concentration is less than pH 3.6.

High concentrations of phosphate tend to produce chlorosis.

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EFFECTS OF VARIOUS METHODS OF APPLYING FERTILIZERS ON CROPS AND ON CERTAIN SOIL CONDITIONS¹

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One of the most important problems in American agriculture today is the correct usage of commercial fertilizers. The increased tonnage of fertilizers sold in the eastern and southern states and the marked benefits from various materials on western soils, indicate definitely that the use of commercial fertilizers in the United States is destined to grow rapidly. With their use, however, comes the question of the proper method of application.

The two common methods employed in the distribution of fertilizers are by broadcasting and by applications at the hill or along the drill-row. Most farmers prefer the latter localized method as it avoids a separate operation and often gives more profitable increases in crop yields. But it may frequently result in injury to seed germination and to plant growth, as is apparent from many studies. Some of the potato and cotton planter manufacturers are attempting to eliminate the danger of this burning effect of fertilizers by locating them above the seed with soil interposed, below the seed, at the sides, or rather thoroughly mixed with the soil in the seed row. It has been noted that plants vary among themselves as to this injury too, the legumes and cotton being much more sensitive to fertilizer burning than the cereals.

Although there is some diversity of opinion as to the nature of the injury, several investigators have concluded that it is due to the increased osmotic pressure of the soil solution and to the consequent retardation in absorption of water by the seed. Some have found a relationship between the inhibited germination or retarded plant growth and a stimulation in the growth of fungi brought about by the fertilizer. A caustic or burning effect of certain fertilizers on the seed or plant roots has been noted in many tests. The injury to plant growth from hill fertilization has also been attributed to drought brought about by an over-luxuriant vegetative growth, which in-

¹ Part I of a thesis (without historical section, bibliography and photographs) presented to the faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² The writer wishes to thank Dr. P. E. Brown for his kindly interest and helpful advice during the progress of this work. Indebtedness to Dr. Paul Emerson, Prof. L. W. Forman, and Prof. L. C. Burnett, for their assistance and timely suggestions, is also gratefully acknowledged. The establishment, by the National Fertilizer Association, of the fellowship which made possible this research has likewise been deeply appreciated.

creases the water requirements of the plants. Restricted root development may in part be the cause of growth injury.

It has been shown that the climatic or seasonal condition, especially the rainfall, is one of the most important factors influencing the occurrence of injurious effects from fertilizers. The concentration or size of application is always of large significance as is also the composition of the fertilizer, the greatest injury occurring when the more soluble chemical fertilizers are used. This fact becomes all the more important when it is noted that the trend of the fertilizer industry is toward chemical mixtures, as for example. the nitrate plants built to make air-nitrates.

The effect of soil type and the movement of salts in soils have been studied by several investigators, and varying results have been secured. The rate of diffusion certainly plays a large part in determining the effects of fertilizers.

EXPERIMENTAL

The experimental work reported in the following pages involved studies of the effects of numerous fertilizers upon seed germination, upon plant growth, and upon certain soil conditions, with various methods of applying the fertilizer relative to the drill-row and hill. Studies of root spread and fertilizer movement through soils watered in several ways were also conducted. The tests were all hand planted both in the field and in the greenhouse.

Field germination tests

These tests were conducted on the Agronomy Farm and various fertilizers were compared as to their toxicity upon corn germination, when applied in direct contact in the drill-row at planting, direct contact in the hill, below the row, below the hill, above the row, and at the side of the row. Tables 1, 2, 3, 4 and 5 give the outlines of the respective tests and the data secured. Germination counts were made at intervals of several days but only the first and last counts are given.

In all of the *row* studies, the fertilizer application was tested in a row $3\frac{1}{2}$ feet long with 1-foot intervals between and 20 kernels planted for each treatment. The *hill* treatments comprised 5 hills of 10 kernels each, except in the *below the hill* studies where 25 kernels represented a perfect stand.

The fertilizer applications were based upon hills 42 inches square and required 12.757 gm. per hill, equivalent to 100 pounds per acre. A tin box 2 inches wide by 3 inches long with the bottom removed, served to make the applications uniform in surface spread and depth of planting in all of the hill studies. The row tests received each fertilizer as a strip 1-inch wide. The commercial 2-12-2 used in all of these tests was Armour's Big Crop brand. The chemical 2-12-2 was home-mixed, using nitrate of soda, acid phosphate, and muriate of potash. The soil type was Carrington loam, a dark brown upland soil of rather high productivity and extensively found in the Wisconsin drift area of Iowa.

The rainfall interfered very markedly with the germination results and undoubtedly there was less injury than would have been found under conditions of continuous dry weather. Nevertheless, 16 per cent acid phosphate retarded germination for all of the rates used in the direct contact in the row

TABLE 1

Germination counts of corn hand drilled and fertilized direct contact in the row*

FERTILIZER	ACID PHO (16 PER			ERCIAL 2-2		EMICAL 2-12-2		XED 12-2
APPLICATIONS RATE PER ACRE		Day	s after pla	nting on v	which the co	unts were n	nade	
	8	15	8	15	8	15	8	15
pounds					_			
Check	17	20	17	20	17	19	16	19
100	8	20	10	17	1	19	2	20
150	4	20	1	14	1	20	2	19
200	7	19	0	5	0	20	0	19
250	2	18	0	8	0	15	0	15
300	1	14	0	4	0	15	0	13
350	0	12	0	3	0	13	0	11
400	0	13	0	1	0	7	0	12
450	0	8	0	2	0	9	0	6
Check	16	20	18	19	14	19	16	20
	ROCK PE	IOSPHATE			NITRATE	OF SODA	MURIATE (
RATE PER ACRE	Days after planting on which counts were made		RATE PER ACRE		Days after planting on which the counts were made			
	8	15		-	8	15	8	15
pounds		****	pour	nds -				
Check	19	19	Che	eck	20	20	18	20
100	19	19		25	14	20	2	20
150	20	20		50	5	19	1	19
200	19	20	7	5	0	17	0	14
250	18	20	10	00	0	14	0	4
300	19	19	12	25	0	6	0	1
400	20	20	15	50	0	5	0	1
500	20	20	17	5	0	1	0	2
	19	20						

^{*} Twenty plants per 3½ feet of row represent a perfect germination.

Check

studies shown in table 1. It failed to show prevention of germination, however, until 300 pounds per acre was applied, when a 70 per cent stand was secured. Of all of the fertilizers tested, it was the least toxic in its effect upon corn germination, except of course raw rock phosphate. The other fertilizers all injured germination, varying considerably as to the limits of

Check

19

20

their toxicity. The commercial 2-12-2 gave pronounced injury at about 200 pounds per acre. In fact, this rate reduced the germination to 25 per cent of a perfect stand.

The applications of nitrate of soda alone and of muriate of potash alone produced the greatest injury. All rates tested, even 25 pounds per acre, gave a retarded germination. The respective breaking points, or rates at

TABLE 2

The effect of various fertilizer locations relative to the seed in the row upon corn germination

	1 INCH BE	LOW SEED	1 INCH AB	OVE SEED	1 INCH SID	E OF SEED
PERTILIZER APPLICATIONS RATE PER ACRE		Days afte	r planting on wh	ich the counts w	vere made	
	10	20	10	20	10	20
		N	itrate of soda			
pounds						
Check	20	20	20	20	14	20
100	0	1	18	18	12	18
150	0	2	14	18	17	19
200	0	1	15	16	16	18
250	1	1	8	13	18	20
300	0	1	7	12	9	19
350	0	0	9	9	17	19
400	0	0	4	7	19	20
450	0	1	2	2	14	18
Check	19	20	20	20	16	19
		Con	ımercial 2–12-	-2		
Check	19	19	18	18	20	20
100	15	20	20	20	20	20
200	17	20	19	20	19	19
300	9	17	19	20	19	20
400	12	16	20	20	18	19
500	1	11	20	20	20	20
600	0	6	20	20	17	18
700	0	2	19	19	19	20
800	0	0	18	19	16	19
Check	19	20	19	20	20	20

which actual inhibition of germination occurred, were 125 and 100 pounds per acre. The fact that 0.84 inches of rain fell just 3 days after planting, should be emphasized, for the breaking points were undoubtedly raised by 50 pounds or more. At the 100-pound rate, the muriate permitted only a 20 per cent stand.

Where the fertilizers were applied 1 inch below, 1 inch above, and 1 inch to one side of the seed row (table 2), the upward movement of the soluble

fertilizer salts through the soil and the injury to corn germination are very apparent, especially in the case of nitrate of soda. Even the lowest rate tested-100 pounds per acre-gave complete inhibition. The commercial 2-12-2 showed the same tendency, only to a lesser degree. The above treatments permitted good germination with the commercial 2-12-2, but in-

TABLE 3 Germination counts of corn fertilized in the hills*

	Germ	ination counts	oj corn jerni	ized in the hi	165			
	ACID PHOSPHATE	(16 PER CENT)	COMMERCI	AL 2-12-2	CHEMICA	L 2-12-2		
PERTILIZER APPLICATIONS RATE PER ACRE Pounds Check 20 40 60 80 100 120 140 160 Check		Days after	olanting on wh	ich the counts w	ere made	re made		
	9	15	9	15	9	15		
pounds								
Check	27	47	47	49	44	46		
20	23	50	17	45	38	47		
40	25	46	2	19	23	46		
60	24	41	0	6	11	39		
80	21	31	0	0	5	31		
100	11	18	0	0	0	13		
120	9	17	0	0	0	4		
140	1	7	0	0	0	0		
160	0	1	0	0	0	0		
Check	36	43	47	47	46	47		
		NITRATE OF SO	DA	8	ULPATE OF AMM	ONIA		
RATE PER ACE	le l	Days aft	er planting on	which the count	s were made			
		9	15	9		15		
pounds								
Check	4	17	47	47		48		
5		7	48	41		50		
10		8	44	24		50		
20		0	42	8		46		

RATE PER ACRE	Da	ys after planting on wh	ich the counts were ma	de
	9	15	9	15
pounds				
Check	47	47	47	48
5	17	48	41	50
10	8	44	24	50
20	0	42	8	46
30	0	22	2	41
40	0	9	2	33
50	0	6	0	17
60	0	0	0	5
75	0	0	0	1
Check	46	48	45	48

^{*} Fifty plants represent a perfect germination.

hibited germination with the high applications of the nitrate of soda. This was very likely due to the downward diffusion of the nitrate, even under the prevailing conditions of dry weather.

Absolutely no injury to germination, either retardation or inhibition, was detected from any of the fertilizers applied along the side of the seed row with soil interposed. These results are in agreement with the writer's previous findings in New Jersey for both rainy and dry seasons.

TABLE 4

Germination counts of corn fertilized 1 inch below seed in the hills*

	ACID PHOSPHATE	(16 PER CENT)	COMMERCI	AL 2-12-2	NITRATE	OF SODA
FERTILIZER APPLICATIONS RATE PER ACRE		Days after p	lanting on wh	ich the counts w	ere made	
	9	15	9	15	9	15
pounds			~			
Check	24	25	25	25	25	25
100	12	24	15	25	18	24
150	21	25	15	25	2	23
200	24	25	11	24	0	23
250	23	25	13	23	0	11
300	16	24	9	22	0	8
350	21	24	5	17	0	7
400	8	21	0	18	0	0
450	5	18	0	15	0	1
Check	25	25	25	25	23	25

^{*} Twenty-five plants represent perfect germination.

TABLE 5

Effect on corn germination of varying the width of fertilizer strip, located 1 inch below the seed in the row*

FERTILIZER TREATMENTS			WID	TH OF FE	TILIZER	STRIP	
		1-1	nch	2-1	nch	4-i	inch
Kind	Rate per acre	Days	after pla	nting on	which co	unts were	made
		9	15	9	15	9	15
	pounds						
4 -: d - b b - to (16)	200	17	20	18	20	20	20
Acid phosphate (16 per cent)	400	18	20	19	19	20	20
Chemical 2-12-2	200	14	20	17	18	19	20
Chemical Z-1Z-Z	400	12	15	14	17	14	20
Nitrate of soda	200	1	3	0	7	2	12
Nitrate of soda	400	0	0	0	2	0	4

^{*} Twenty plants represent a perfect germination.

In table 3, the effects of *direct contact in the hill* applications of fertilizers are shown. The results of an additional test with acid phosphate and commercial 2-12-2 are not given, as they were quite similar to those presented.

Again acid phosphate proved the least injurious of the various fertilizers tested. However, its breaking point was around 100 pounds per acre, or much lower than when applied in *direct contact in the row*.

The commercial 2-12-2 gave a pronounced injury and its breaking point was between 20 and 40 pounds per acre. Applications of 80 pounds and more completely prevented germination. This occurred under conditions of high moisture content in the soil too, for it rained 0.76 inch just two days prior to the planting. The nitrate of soda and the sulfate of ammonia also gave badly injured corn germination. The breaking point of the former was between 30 and 40 pounds per acre, whereas for the latter it was about 60 pounds.

From these results it appears that all fertilizer attachments permitting the direct contact in the hill location of fertilizers are very undesirable for use.

The effects upon corn germination of three fertilizers when located 1 inch below the hill are given in table 4. Rainfall on the day following the planting and three days later certainly affected the results; nevertheless, the injury from the nitrate of soda was marked. It showed a breaking point of 250 pounds per acre, at which application there was a 40 per cent germination. The acid phosphate and the commercial 2-12-2 gave no positive inhibition of germination, but retarded the germination for all of the rates tested. The below location of fertilizers evidently will not eliminate the dangers to seed germination.

Table 5 shows the results of a study of the effects upon germination of varying the width of the fertilizer strip located 1 inch below the seed row. In general it was found that the wider the strip for a given fertilizer at a given depth below the seed in this soil, the less was the injury to germination. This fact was again shown best by the nitrate, but the chemical 2-12-2 also indicated it. The acid phosphate failed to give any noticeable retardation of germination for the various rates of application tested.

If below methods of fertilizer distribution are used, provision should certainly be made for adjusting the area of spreading the fertilizer or the width of its strip and the depth below the seed-row or hill. But it has not been shown conclusively that there is no danger to root development and seed-ling growth from large applications of concentrated soluble chemical fertilizers located below the hill or the drill-row and hence the method is not recommended. The results do seem to indicate, however, that side locations of the fertilizer are safe to germination and effective in benefits.

Greenhouse studies

Some row and hill fertilization studies were made under control conditions in the greenhouse. Wheat and oats were grown in benches with row applications of the commercial 2–12–2 fertilizer, applied in treatment-lengths of 1 and 5 feet respectively. On the basis of these treatment-lengths and with a 7-inch interval, the wheat received 2.43 grams whereas the oats

received 12.15 grams per 400 pounds per acre application. The Ammo-Phos plus KCl treatment used on the oats was applied at $\frac{1}{4}$ the rate of the 2-12-2. Tables 6 and 7 give the respective outlines of the tests and the dry harvest weights after 10 weeks' growth.

TABLE 6
Greenhouse study of methods of fertilizing wheat

TREAT- MENT NUMBER	METHODS OF APPLYING THE FERTILIZER RELATIVE TO THE SEED ROW	DRY HARVEST WEIGHTS PER TREATMENT FOR THE FOLLOWING RATES OF FERTI- LIZER APPLICATION IN FOUNDS PER ACRE					
NUMBER	(COMMERCIAL 2-12-2)	Check (none)	200 pounds	400 pounds	600 pounds	800 pounds	
		gm.	gm.	gm.	gm.	gm.	
1	One side of row, 1 inch away, same plane	18.0	21.0	20.5	23.0	25.5	
2	Both sides of row, split, 1 inch away, same plane.	15.0	19.5	21.0	21.5	23.0	
3	Both sides of row, split, ½ inch away, same plane	16.0	19.0	22.0	23.0	26.0	
4	Both sides of row, split, 1 inch away, 1 inch lower plane	20.0	22.5	26.0	26.0	29.0	
5	1 inch below seed row	17.5	25.0	23.5	27.0	28.0	
6	inch below seed row	22.0	27.0	29.5	33.0	32.0	
7	inch above seed row	21.0	20.0	24.0	27.0	25.0	
8	Direct contact in seed row	24.0	33.0	42.0	46.0	37.5	

TABLE 7
Greenhouse study of methods of fertilizing oats

		DRY HARVEST WEIGHTS (10 WEEKS' GROWTH)			
TREAT- MENT NUMBER	METHOD OF APPLYING THE FERTILIEER RELATIVE TO THE SEED ROW	Commercial 2-12-2 (400 pounds per acre)	Ammo-Phos + KCl (100 pounds per acre)		
		gm.	gm.		
1	Check—unfertilized	176	164		
2	Direct contact in seed row	195	182		
3	One side of row, 1 inch away, same plane	200	191		
4	1 inch below seed row	205	197		
5	1 inch above seed row	190	169		
6	Check—unfertilized	180	158		

The direct contact fertilization with rates of application tested, allowed a very satisfactory growth of the wheat. The high rates, however, proved somewhat depressing to growth. Undoubtedly, the frequent waterings decreased the injury so that under normal field conditions a safe germination and good growth could be expected only at much lower rates. The above fertilization

gave small benefits, but they were probably larger than would have been realized in the field in a dry season. The below and sides locations of the fertilizer gave fair responses, but were not so effective as the direct contact.

With the oats the *direct contact* fertilization brought about increases in growth, but the *below* and the *side* applications gave the largest beneficial effects. Again the *above* location of the fertilizer led to small yields. The results were similar for both fertilizers tested. It appears from these results that moderate applications of fertilizers distributed *direct contact* in the seed rows will give very satisfactory results for cereals like wheat and oats, which are very resistant to fertilizer burning.

TABLE 8
Greenhouse study of hill locations of fertilizer on corn

TREAT- MENT NUMBER	FERTILIZER LOCATION BELATIVE TO HILL®	AVERAGE PLANT HEIGHTS	DRY HARVEST WEIGHTS
		inches	gm.
1	1 inch above hill, strip 1 inch by 4 inches	40	43
2	1 inch below hill, strip 1 inch by 4 inches	50	52
3	Check—unfertilized	45	40
4	One side of hill, 1 inch away, same plane, strip 1 inch by 4 inches	65	67
5	Rear of hill, 1 inch away, same plane, strip 1 inch by 4 inches.	60	43
6	Both sides of hill, split, 1 inch away, same plane, strip 1 inch by 4 inches.	70	57
7	Check—unfertilized	50	42
8	Both sides of hill, split, 1 inch away, 1 inch lower plane, strip 1 inch by 4 inches	70	56
9	Band around hill, 1 inch away, same plane, 1 inch width	60	48

^{*} Two hills per treatment, each receiving 12.75 grams of Commercial 2-12-2, which is equivalent to 100 pounds per acre.

In the greenhouse study of corn fertilization reported in table 8, Reid's Yellow Dent seed was planted with 4 kernels per hill. The crop was harvested at two different periods of growth, but only the final harvest yields are given with the average plant heights, as the results were very similar at the two harvests.

The results indicate that the sides of hill location for the fertilizer is beneficial and certainly equal to any of the other methods tested. Both the sides and the below locations gave results superior to the rear of hill and the above the hill positions.

Field fertilizer studies—hill methods

Table 9 outlines a test of hill methods of applying the fertilizer, which was planted in duplicate on the Agronomy Farm. Ammo-Phos plus muriate of potash was used. Two days after planting, 2.10 inches of rain

fell, which aided the above the hill fertilization and reduced the injury from the below locations. Nevertheless, the high applications below gave considerable injury and the above applications failed to equal the small below or both sides applications.

A study of the effects of area of spread of the fertilizer below the seed in the hill was also made. The results show that increased area of spread and increased depth of locating the fertilizer serve to safe-guard the seed germination and the seedling growth.

TABLE 9
Field study of hill methods of applying the fertilizer

TREATMENT		FERTILIZER APPLICATIONS	DRY
NUMBER	Per hill	Location relative to hill	WEIGHTS
	gm.		pounds
1	9.18	1 inch below, 2 inches by 5 inches spread	7.5
2	18.36	1 inch below, 2 inches by 5 inches spread	4.0
3	18.36	1 inch below, 4 inches by 5 inches spread	7.1
4	18.36	1 inch above, 4 inches by 5 inches spread	6.5
5	None	Check	1.5
6	18.36	1 side, 1 inch away, 2 inches by 5 inches spread, same plane	6.0
7	18.36	Both sides, split, 1 inch away, 2 inches by 5 inches spread, same plane	7.0
8	18.36	Band around hill, 1 inch away, same plane	6.7
9	18.36	Both sides, split, 2 inches by 5 inches spread, 1 inch lower plane	7.4
10	36.72	1 inch below, 2 inches by 4 inches spread	1.1
11	36.72	1 inch below, 4 inches by 4 inches spread	4.5
12	36.72	1 inch below, 6 inches by 6 inches spread	6.3
13	36.72	2 inches below, 4 inches by 4 inches spread	7.0
14	36.72	2 inches below, 6 inches by 6 inches spread	7.5

Field fertilizer studies—row methods

This study of the various locations of fertilizer along the seed row, was made at the Agronomy Farm. Each row (except the checks) consisted of 6 fertilized treatments with increasing rates of application. The Ammo-Phos plus KCl was again used, the treatments being 16 feet long and having 50 corn kernels each. Border rows were run on all sides of the test. Table 10 gives the plan of the test with the final immature harvest weights. Weights were taken at one earlier date but the results are omitted as they were quite similar. Fifteen plants were harvested on each date.

A depression in germination for all of the *below* fertilized rows was noted, no. 4 being more retarded than no. 3 because of the narrow spread of the fertilizer located 1 inch below the seed at the planting. All of the above fertilized rows evidenced good growth, since rains occurred to leach the soluble salts down around the roots but not soon enough after planting to

injure the germination. Six days after the test was started, 1.15 inches of rain fell, followed five days later by 1.25 inches.

All of the *side* treatments gave good growth throughout the season with no retardation of germination and no depression of early growth. If the season had been dry, however, the *above* fertilized rows would undoubtedly have failed to give as good response. The great solubility of the fertilizer

TABLE 10
Field study of row methods of applying fertilizer

		FINAL	GERMIN		HAR	FINAL VEST WEI	GHTS
ROW	FERTILIZER LOCATIONS RELATIVE TO SEED ROW			For ferti	lizer rate	s of	
NUMBER	RELATIVE TO SEED ROW	100 pounds	200 pounds	400 pounds	100 pounds	200 pounds	400 pounds
					pounds	pounds	pounds
1	Check—unfertilized	48	49	50	4.7	5.0	4.1
2	2 inches below, narrow spread	49	50	39	9.0	11.0	12.4
3	1 inch below, wide spread	48	47	37	11.5	12.6	13.0
4	1 inch below, narrow spread	49	44	31	10.0	10.7	11.0
5	1 inch above, narrow spread	50	49	42	10.7	11.5	12.5
6	1 inch above, wide spread	50	49	46	11.4	11.8	12.7
7	1 inch above, split as with "deflectors"	48	47	50	11.0	12.4	13.0
8	Check—unfertilized	49	50	49	4.9	5.5	3.9
9	Both sides, 1 inch away, narrow spread,						
	same plane	49	49	50	12.0	12.6	13.3
10	Both sides, 1 inch away, wide spread,						
	same plane	48	50	48	10.8	11.5	12.5
11	Both sides, 1 inch away, narrow spread,						
	lower plane	47	48	49	10.5	11.0	12.1
12	One side, 1 inch away, narrow spread,						
	lower plane	50	48	46	10.4	10.8	11.5
13	One side, 1 inch away, narrow spread,						
	same plane	50	48	47	11.5	11.5	12.0
14	One side, 1 inch away, wide spread,						
	same plane		50	.49	10.7	10.5	11.0
15	Mixed with soil in seed row	47	42	27	9.0	10.3	7.5
16	Check—unfertilized	1	48	49	5.2	6.4	5.5
17	Direct contact with seed in row	30	7	0	6.4	1.5†	None

^{*} Twelve plants harvested.

mixture aided the above rows tremendously too. The data in the table again show the bad effects of high applications of concentrated, readily-soluble chemical fertilizers distributed direct contact in the seed rows or mixed with the soil in the seed row.

Root growth study

In the root growth study, corn was planted in the center of wire baskets in 4-gallon pots containing a loam and allowed to grow with the roots inter-

[†] Two plants harvested.

twining with the mesh of crosswires of the baskets. Hence, the roots were retained in their normal growth directions after the supporting soil had been washed away. Various locations of the commercial 2–12–2 relative to the hills were then made at the rate of 200 pounds per acre and different methods of watering the pots were followed.

A decided outward growth of the roots was found in all of the baskets where the fertilizer zone was located directly below the hill. This tendency was greater in the capillary watered pots than in the surface watered ones. The high concentration of the salts probably explains the negative growth

TABLE 11

Series I: Greenhouse study of phosphorus movement in a loam soil with irregular surface

watering of the boxes

BOX NUMBER AND FERTILIZER TREATMENT	LOCATION OF SAMPLES	AVERAGE TOTAL PHOS- PHORUS IN SOIL	BOX NUMBER AND FERTILIZER TREATMENT	LOCATION OF SAMPLES	AVERAGE TOTAL PHOS- PHORUS IN SOIL
1. (0-16-0), rear of {	Above Below Sides	per cent 0.134 0.334 0.131	6. (3-12-3), rear of { hill	Above Below Sides	per cent 0.137 0.217 0.125
2. (0-16-0), broad- {	Surface soil Subsoil	0.260	7. (3-12-3), broad- {	Surface soil Subsoil	0.195 0.137
3. (0-16-0), 1 inch below hill	Above Below Sides	0.133 0.283 0.137	8. (3-12-3), 1 inch below hill	Above Below Sides	0.134 0.195 0.129
4. (0-16-0), 1 inch above hill	Above Below Sides	0.139 0.318 0.135	9. (3-12-3), 1 inch { above hill	Above Below Sides	0.145 0.276 0.126
5. (0-16-0), sides of { hill	Above Below Sides	0.134 0.375 0.137	10 (3-12-3), sides of hill	Above Below Sides	0.135 0.197 0.130

movement. With applications of acid phosphate located below the hill, no injurious root growths were found and only a stimulated development appeared. The tendency toward restriction and negative movement seems to be associated with the concentrated soluble chemical fertilizers and occurs primarily during the first few days of the seedling growth.

Fertilizer diffusion studies

Two series of fertilizer diffusion studies were run in the greenhouse. The first one was conducted with 10 cropped boxes and the second one with 6 uncropped boxes. Various hill locations of acid phosphate and a complete 3–12–3 fertilizer were employed in the different boxes at 300 pounds per

acre, but with the same kind of hill location in all of the compartments of a given box. The cropped boxes grew corn. All of the boxes in the first series were surface watered, but in the second series 2 soil types and 3 systems of watering were used. The general outlines of the series are shown in tables 11 and 12, which also give the results.

The soil samples on which these tests were made, were secured 1, 2, 3, 4, 6, 8, 10 and 12 weeks after starting the studies in series I, and 4, 11, 18, 25, 32,

TABLE 12

Series II: Effects of soil type and methods of watering the boxes upon phosphorus diffusion

BOX NUMBER AND SOIL TYPE	METHOD OF WATERING THE BOXES	LOCATION OF SOIL SAMPLES RELATIVE TO FERTI- LIZERS	AVERAGE TOTAL PHOS- PHORUS IN SOIL	AVERAGE TOTAL NITROGEN IN SOIL
			per cent	per cent
4 77 1 . 1		Above	0.094	0.420
1. Webster loam	Surface	Below	0.418	0.559
		Sides	0.099	0.410
		Above	0.099	0.435
2. Webster loam	Combination	Below	0.163	0.467
		Sides	0.104	0.408
		Above	0.171	0.443
3. Webster loam.	Capillary	Below	0.126	0.425
		Sides	0.100	0.415
		Above	0.075	0.240
4. Miami silt loam	Surface	Below	0.294	0.316
		Sides	0.072	0.233
		Above	0.107	0.254
5. Miami silt loam	Combination	Below	0.178	0.295
0.0000000000000000000000000000000000000	Combination	Sides	0.093	0.241
		Above	0.194	0.268
6. Miami silt loam	Capillary	Below	0.095	0.247
Vi atanuari date average, e	Cupillary	Sides	0.089	0.235
		(Dides	0.009	0.200

^{39, 46} and 53 days after, in series II. They were taken from a new compartment in each box and in definite locations relative to the respective fertilizer zones. Only the average results are given. In the broadcast boxes, only two samples were taken, one from the surface soil, the other from the subsoil which received no fertilizer. In all the hill fertilized boxes, three soil samples were taken as follows:

⁽a) The soil layer from $\frac{1}{2}$ to $1\frac{1}{2}$ inches directly above the fertilizer zone; (b) the same soil layer directly below the zone; and (c) the soil on all sides of the fertilizer zone from $\frac{1}{2}$ to $1\frac{1}{2}$ inches away horizontally.

A study of the tables shows the following facts:

(a) The soluble phosphorus fertilizer moved downward with the surface watering. (b) Likewise, it moved upward with the capillary watering. (c) The downward leaching movement was more pronounced than the upward capillary movement. (d) The most rapid and the greatest movement took place in the above the hill fertilized boxes. (e) The least rapid and weakest movement occurred in the below the hill fertilized boxes. (f) The larger part of this movement took place immediately following the starting of the studies. In fact, the maximum was frequently indicated for the soil sampled first, with a gradual decline in the percentage found in the later samples. (g) The movement of nitrogen was found to be similar to that of phosphorus. (h) At least half or more of the original fertilizer applications remained at the conclusion of the studies in their respective zones. This fertilizer appeared granular and identical with the original material, only of course the soluble salts had been largely leached away.

CONCLUSIONS

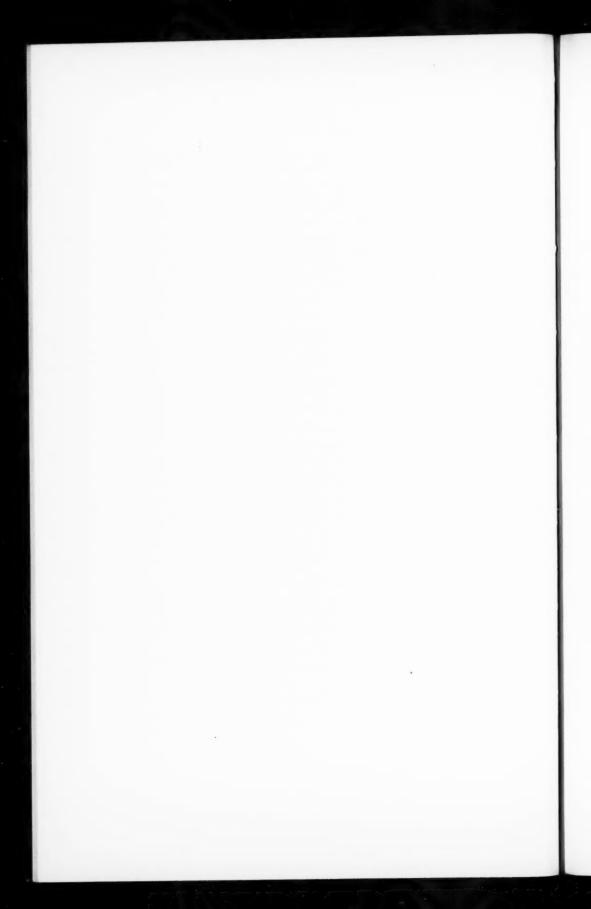
A general study of the data presented shows first of all that fertilizer applications located direct contact in the hill or direct contact in the drill-row with seed are likely to be very injurious to the best germination of the seed. Various factors, such as rainfall, soil type, kind of seed, and kind of fertilizer obviously modify the injury obtained. Very small amounts of concentrated, readily-soluble chemical fertilizers are injurious, and these amounts are often smaller than the rates for which the fertilizer attachments upon the present day seeding machines are capable of adjustment.

The low applications of fertilizers give retarded germination, but the high ones cause inhibition of germination. The injury increases directly with the fertilizer application. Therefore, for the best germination of all seeds under all conditions of climate and soil, the distribution of fertilizers direct contact either in the hill or in the row with the seed is not advised. Those planters with fertilizer attachments so designed, either to cause or to permit this direct contact application of the fertilizer should certainly be redesigned.

In place of the direct contact method of fertilizer application, there are several other methods of localized distribution. The above the hill or drill-row, the below the hill or drill-row, and the sides of hill or drill-row methods seem most promising. Because of a direct dependence of the first two methods upon climatic conditions and other variable factors, the above and the below locations are felt to be unwise. Therefore, the sides method of fertilizer distribution remains as the best solution of the problem for all crops sown in hills or rows with wide intervals between the successive rows. Horizontal diffusion of salts is very slow, and hence the sides method proves safe to germination. Then too, the fertilizer is located in the immediate proximity of the young seedling's branching roots to furnish immediately sufficient plant-food to permit of a good crop yield and early maturity.

As another possible method of such localized fertilizer distribution—a method which is certainly better than the *direct contact* location but not entirely free of dangers to germination—the *mixed with the soil in the hill or*

drill-row method is suggested. From the standpoint of localized fertilization to promote the best growth, this method should be almost ideal. However, with very high applications of concentrated, readily-soluble chemical fertilizers, the method will cause marked germination injury. As long as moderate amounts of non-caustic fertilizers are applied by this method through an efficient attachment, good results should be secured. If the limitations of the method could be accurately understood by all parties concerned and if farmers would be willing to split their large fertilizer applications between localized and broadcast methods, then the mixed in the row method might be considered satisfactory. Under the existing conditions, however, the sides method alone is recommended for localized fertilizer applications.



POTASSIUM FERROCYANIDE AND FERRIC FERROCYANIDE AS SOURCES OF IRON FOR PLANTS

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In search of a compound that would furnish iron satisfactorily to plants-grown in weakly acid or slightly alkaline nutrient solutions a number of experiments were performed with potassium ferrocyanide and ferric ferrocyanide.¹ Potassium ferrocyanide is unique as an iron source because it contains iron in the anion. Ferric ferrocyanide, or Prussian blue, contains iron in both the cation and anion and is relatively insoluble in water. These complex iron salts were found to be fair sources of iron for green plants under rather limited conditions. With potassium ferrocyanide the chief condition was to secure a low enough concentration of the salt to escape its toxic effects, whereas with ferric ferrocyanide the reaction of the nutrient solution was the chief factor determining the availability of the iron.

Knop (1) in 1869 found that chlorotic corn plants became green when supplied with potassium ferrocyanide at the rate of 0.1 per mille (13.22 p.p.m. of iron) but that the growth of the plants was slowed and finally stopped. Wagner (8) in the following year confirmed the results of Knop and observed a slight deposit of ferric ferrocyanide on the roots. Susuki (5) observed a poisonous action on barley seedlings of potassium ferrocyanide at a concentration of 0.01 per mille (1.322 p.p.m. of iron) in water cultures. In soil cultures (6) it stimulated growth. He ascribed the poisonous action to hydrocyanic acid, which was formed within the plant by the splitting of the potassium ferrocyanide, but was unable to decide whether the stimulating action in the soil was due to the compound itself or to its decomposition products. Loew and Kozai (2) observed a stimulating effect of 0.01 per cent potassium ferrocyanide on Bacillus prodigiosus but no improvement in the growth of other bacteria. No reference to the use of ferric ferrocyanide as a source of iron for plants was found in the literature.

EXPERIMENTAL

Spirodela polyrhiza (L.) Schleid. plants, floating aquatics, were grown in Knop's nutrient solution diluted 10 times in accordance with the findings of Saeger (4). Iron was added to the solution in the forms of ferric citrate,

¹ The author wishes to acknowledge his thanks to Dr. W. J. Robbins for helpful criticism and advice in the course of this investigation.

ferrous sulfate, ferric chloride and potassium ferrocyanide at the concentrations indicated in table 1. To one solution no iron was added.

The three commonly used iron salts: ferric citrate, ferrous sulfate, and ferric chloride produced a very satisfactory growth of these plants, the largest growth being secured with ferrous sulfate as is shown in table 1. The leaves of the plants in the solution containing the lowest concentration of iron as potassium ferrocyanide (0.016 p.p.m. iron) were small and chlorotic. The plants in the solutions containing 0.033 and 0.066 p.p.m. iron as potassium ferrocyanide made fair growth but the leaves were a trifle lighter green than those of plants in the solutions containing the commonly used iron salts.

TABLE I

Growth data of Spirodela plants grown 23 days in Knop's solution containing iron in the salts
as the concentrations indicated.*

	CONCENTRA-	AVERAGE NUMBER MATURE	AVERAGE GREEN WEIGHT	REACTION	OF SOLUTIONS
IRON SALT	TION OF IRON	LEAVES PER CULTURE	PLANTS PER CULTURE	Initial	Maximum final
_	p.p.m.		mgm.	фH	pΗ
Check	0.000	18.25	37.00	6.6	7.4
Fe citrate	2.280	152.25	513.75	6.4	7.3
FeSO ₄	3.676	160.75	563.75	6.5	7.1
FeCl ₂	2.065	137.25	527.00	6.2	7.0
(0.016	62.00	140.00	6.7	7.4
	0.033	102.00	275.00	6.7	7.6
K4Fe(CN)6	0.066	93.50	308.75	6.7	7.6
	0.132	78.50	267.25	6.7	7.7
[0.264	53.50	171.25	6.6	7.5

^{*} The nutrient solutions were renewed 5 times at intervals of 4 days.

With the highest concentrations of iron as potassium ferrocyanide (0.132 and 0.264 p.p.m. iron) the leaves of the plants were normal green but the growth of the plants with the latter concentration was greatly reduced. The plants in the solution without iron made very poor growth and the leaves were chlorotic.

With soybean plants concentrations of 0.264 p.p.m. and higher of iron in the form of potassium ferrocyanide greatly depressed growth. Concentrations of 0.033 and 0.066 p.p.m. iron in this iron salt produced fair growth of soybean plants, but chlorophyll development was not adequately provided for, the leaves being light green.

Potassium ferrocyanide was used in solutions having reactions from pH 3.1 to 8.3. Spirodela plants grew best at pH 6.2 and 6.8. This iron salt was not so satisfactory at neutral and slightly alkaline reactions as ferric citrate.

Merck's iron ferrocyanide used at concentrations of 0.022 to 9.0 p.p.m. iron per liter in Knop's solution resulted in very poor growth and chlorosis of the

[†] Average of 4 cultures.

foliage of soybean and Spirodela plants. The explanation of these results was found when this iron salt was used in a series of buffered solutions having reactions of pH 4.0 to 8.3. Knop's solution was modified by omitting KNO₃, substituting H₃PO₄ at the rate of 0.8055 gm. per liter for KH₂PO₄, and adding potassium acid phthalate at the rate of 1.0207 gm. per liter. These changes were based on the buffered nutrient solution developed by Tarr and Noble (7). At pH 5.0 soybean plants made a greater growth when the solution contained ferric ferrocyanide than when it contained ferric citrate, the iron contents of both solutions being the same (5 p.p.m. iron). At pH 5.5 the foliage of the plants in the solution containing ferric ferrocyanide was light green and at all higher reactions it was distinctly chlorotic. The plants in the solutions con-

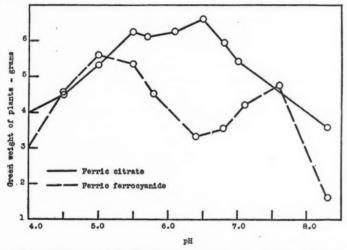


Fig. 1. Green Weight of Soybean Plants for each Culture in Nutrient Solutions Containing 5 p.p.m. Iron as Ferric Citrate and Ferric Ferrocyanide

taining ferric citrate made very satisfactory growth from pH 5.5 to 6.5, but from pH 6.8 to 8.3 the leaves of the plants were chlorotic.

The reaction of Knop's solution as used for soybeans in the earlier experiments was pH 5.8 and when diluted 10 times for *Spirodela* plants, pH 6.6. Both of these reactions were in the region in which the iron of ferric ferrocyanide in the above experiment was insufficiently available for the normal growth of soybean plants.

The growth curve (fig. 1) of the soybean plants grown in the buffered nutrient solutions containing ferric ferrocyanide shows two maxima, one occurring at pH 5.0 and a second at pH 7.6 with a marked depression in growth around pH 6.4. It is of interest to note that Robbins and Scott (3) found the isoelectric point of soybean root tips to be in the vicinity of pH 6.4.

SUMMARY

1. With 0.033 and 0.066 p.p.m. iron in the form of potassium ferrocyanide, soybean and *Spirodela polyrhiza* plants made fair growth. Higher concentrations of iron in this salt produced a slow stoppage of growth.

2. Merck's ferric ferrocyanide was a satisfactory source of iron for soybean plants when the solution had a reaction of pH 5.0 but at less acid reactions growth of the plants and chlorophyll development was restricted.

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THE VIABILITY OF THE NODULE BACTERIA OF LEGUMES OUTSIDE OF THE PLANT: I, II

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INTRODUCTION

The success or failure of the process of inoculation depends upon the viability of the nodule bacteria of legumes outside of the plant. There are many factors which must be taken into consideration in connection with the life or death of the nodule bacteria when removed from their host plant. Some of the important factors controlling their active resistance have been studied from time to time by various investigators. It still remains true, however, that perfectly viable pure cultures are applied to seeds, and an insufficient number of the organisms remain alive on the seed to give satisfactory inoculation before the seed is planted and, in some cases, after the seed has been planted.

Soil inoculation also fails under conditions which can be accounted for in part on the basis of certain important factors which are included in this study. Because of the very common failure of commercial cultures and of the occasional failures of pure cultures put out by experiment station workers, a study of the fundamental factors responsible for the life of the organisms under the conditions to which the seed must be subjected was considered as the first and most important step in the solution of the problem of successful inoculation.

Certain clean-cut, positive data regarding the organism and its growth under proved conditions have been accepted as a basis upon which to build the foundation for this experimental work. A great deal of work irrelevant to the real factors concerned in a successful carrying out of this process has been published in connection with the inoculation problem. The work of Löhnis, Burrill, Whiting, Hansen, Bewley and Hutchinson, Fred, Shunk, and their associates made it possible to outline definite studies dealing with the factors concerned in the viability of the nodule bacteria outside of the plant. The work of Whiting and associates (14) with nodule bacteria covers a decade and

¹ Portion of a thesis submitted to the faculty of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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the writer had gathered the salient points in this experience for attacking this problem. Especially is the work of Löhnis and Smith (10) on the life cycles of bacteria, Löhnis and Hansen (9), and Bewley and Hutchinson (2) pertinent to this investigation.

In some cases, determinations have been made that are repetitions of work found in the literature. These have been made a part of these investigations because it was necessary to establish definitely many points related to the facts brought out by the above workers.

Data for nodule production as influenced by time of storage, temperature during storage, kind of container for storage, maintenance of the organisms in pure and impure cultures in association with other nitrogen-fixing bacteria, with yeasts, molds, and with non-nitrogen-fixing bacteria on and in different media; the effect of different treatments such as the reinforcing of the inoculation with sugar, glue, and soil in various combinations and in different concentrations; the effect of calcium carbonate, tricalcium phosphate, and sugar in various amounts with soil, constitute the first part of this study. The effect of sunlight, dessication, and aeration were investigated. Extensive studies were conducted as to the effect of time and dilution upon the number of the legume organisms surviving when cultured in liquid media; the effect of limited and ample quantities of oxygen upon the life of the organism grown both in liquid and in solid media; and the comparative effect of cane sugar and mannite, both in solid and in liquid media, upon the life of the legume organisms. The effect of acidity in the soil on the infective power of nodule bacteria was studied. Thermal death point determinations were made without reference to previous determinations.

The solution of these problems is dependent in part upon a knowledge of the life cycle of the organisms, and with this in view, the last part of this investigation was devoted to extensive experiments concerning the life cycle of nodule bacteria as influenced by certain chemicals.

HISTORICAL

Beijerinck (1) in 1887 first isolated in pure culture from the nodules of legumes, an organism which he described as *Bacillus radicola*. The description given by Beijerinck represents a fairly accurate description of the nodule bacteria as they are now recognized.

Burrill and Hansen (3) in 1917 proved that the organisms producing nodules on cowpeas and soybeans were typical of *Pseudomonas*, and the name *Pseudomonas radicicola* was properly used by them in describing the organisms concerned with those legumes.

Löhnis and Hansen (9) produced a convenient and well adapted method which greatly assists in proving the purity of the legume cultures.

Hopkins and Burrill (7), Whiting and Hansen (14), and Whiting and others have worked out a number of groups of cross-inoculations and have accumulated a large number of data dealing with the factors responsible for suc-

cessful inoculation. The information obtained by these workers has been available in connection with the present study.

Fred and Davenport (6) have determined the pH values for a number of legume bacteria and Azotobacter, and these values have been found useful in connection with this work.

Shunk (12), working at the North Carolina experiment station, has succeeded in making flagella stains of nodule bacteria from 41 species of legumes. His work is in practically complete agreement with the work published from the Illinois experiment station, and furnishes an important contribution toward the clearing up of the whole question of the flagellation of nodule bacteria and the question of whether there are two organisms concerned in nodule production.

Certain references related to the study of the life cycle of nodule bacteria will be considered under part V of this paper.

PART I—EXPERIMENTS ON THE ENDURANCE OF NODULE BACTERIA IN SOIL AND
ON SEED AS INFLUENCED BY VARIOUS TREATMENTS AND TIME
OF STORAGE BEFORE AND AFTER INOCULATION

Methods Employed

The cultures used were obtained from the pure stock cultures of Doctor A. L. Whiting. Many of the cultures from which the infusions were made were grown in 8-ounce glass bottles. Unless otherwise stated, it is to be understood that only pure cultures of the nodule bacteria were employed in these experiments.

Soybeans, sweet clover, garden peas (wrinkled and smooth), and cowpeas were used in these experiments. The seeds were sterilized in a solution of mercuric chloride (1 to 500) for 10 minutes, after which they were washed with sterile distilled water at least fourteen times, then dried.

Clean quartz sand was placed in pint glass fruit jars and the whole sterilized for several hours in a hot air oven at 350°C. After the jars were cooled and well aerated, 1 gm. of pure calcium carbonate was added to each. The moisture content of the sand was maintained at about 14 to 16 per cent. Usually 7 of the larger seeds and 25 to 30 of the smaller seeds were planted in each jar. The jars, carefully covered with wrapping paper, were left in the laboratory until the seeds had germinated. Then they were kept in the greenhouse until washed out for nodule examination. Plant-food elements with the exception of nitrogen, were applied to all the plants in all the experiments a number of times depending upon the duration of the experiment.

An examination for the presence of nodules was made usually after the peas were 20 days old. The development of nodules varied considerably for different plants; for example, the Alaska peas (smooth), developed nodules in 12 days, whereas the late peas (wrinkled) failed to develop nodules much before

20 to 27 days. An abnormal growth was often found to retard the appearance of nodules.

The data on nodule production are classified as many, few, and none.

Experiment 1—The Effect of the Addition to the Nodule Infusion of Soil, Glue, and Sugar in Different Combinations on the Length of Time the Organisms Survive on the Seed

The purpose of this experiment was to test the length of time that the nodule organisms would survive in sufficient numbers for successful inoculation when applied to the seed as an infusion made with either distilled or tap water. Additions of soil; glue; and soil and glue; sugar; sugar and soil; and sugar, glue, and soil were made to the bacterial infusion and these mixtures applied to the seed. The amounts of the various materials used in making these mixtures are given in table 1.

TABLE 1
Additions made to bacterial infusion in experiment 1

LOT	TREATMENT
1	Bacterial infusion
2	Bacterial infusion + 30 gm. air-dried brown silt loam
3	Bacterial infusion + 15 cc. of glue solution (60 gm. glue for each liter o water.)
4	Bacterial infusion + 30 gm. of soil + 15 cc. of glue solution
5	Bacterial infusion +7 gm. cane sugar
6	Bacterial infusion + 30 gm. soil + 7 gm. cane sugar
7	Bacterial infusion + 30 gm. soil + 15 cc. glue + 7 gm. cane sugar

Each lot of seed weighed 2 pounds and received the bacterial infusion on the basis of 1 bottle for each bushel of seed. The bacterial infusion was applied to 14 pounds of seed, which was thoroughly mixed and divided into 7 lots. Caution was exercised to mix thoroughly all of the various additions with the seeds. Treated lots were dried in enameled pans and then transferred to ordinary cloth seed bags and stored in a locker in the laboratory. After 1 month, each lot was divided into 2 parts, one part being left in the cloth bags and the other part placed in glass fruit-jars with tight tops. The jars were stored in the locker, whereas the bags were stored in the laboratory store room. The humidity and temperature conditions were approximately the same in both places.

In this first experiment, soybeans, sweet clover, and wrinkled garden peas were used. The soybeans were inoculated October 7, the sweet clover, October 9, and the wrinkled peas, October 18, 1920. Duplicate jars of each treatment were planted immediately and after various intervals, as shown in table 2.

The results from all the series of soybeans, sweet clover, and garden peas in this experiment as reported in tables 1, 2, and 3 show that nodule production

was fairly consistent, regardless of conditions and kind of treatment. The number of nodules produced from all treatments would appear to be ample for the fixation of nitrogen for normal growth. As an average, 12 were found on each plant at the various stages. The time of storage after inoculation, up to 2 months, did not materially influence nodule production. Depressions were seen here and there, but in no cases were they important. In such cases, it is believed that uneven distribution of the organisms occurred. This is shown where one lot developed a few nodules at the immediate planting, but developed many nodules at the later plantings. The number of times the seed was mixed would account for certain of these fluctuations.

The treatments containing sugar showed a nodule production superior to the treatments containing soil or glue. With sugar treatments the nodules were uniformly large and evenly distributed over the root system. Besides, the nodule production was fairly consistent through all the plantings (24 hours, 36 hours, 48 hours, 72 hours, 2 weeks, 1 month, and 2 months). The stimulative

TABLE 2
Series and intervals of planting

INTERVALS OF PLANTING		SERIES	
INIBAYADS OF FERRING	Soybean	Sweet clover	Garden peas
Immediately after inoculation	100	1,000	2,080
24 hours after inoculation	200	1,010	2,090
36 hours after inoculation	300	1,020	2,300
48 hours after inoculation	400	2,030	2,310
72 hours after inoculation	500	2,040	2,320
2 weeks after inoculation	600	2,050	2,330
1 month after inoculation	700	2,060	2,340
2 months after inoculation	900	2,070	2,350

action of sugar is probably due to its supplying energy upon which the organisms feed when put under favorable conditions. Sugar possesses the characteristic of gathering moisture from the air, thereby creating on the seed-coat, a film which serves as a protection against the harmful effect of desiccation. Although Clerk (4) stated that moisture has none, or very little effect on the viability of the organisms, yet he failed to show whether its absence is harmful to the organisms under storage conditions. The thin film of moisture on the inoculated seeds treated with sugar, may permit the organisms to move through and distribute themselves more evenly on the seeds.

Soil used with the bacterial infusion was found to be beneficial. The soil particles may serve as a protection for the organisms against desiccation, because of the film of moisture around the soil particles. Soil contains food which helps to prolong the life of the bacteria.

Glue with the bacterial infusion was of very little benefit although it was not found harmful to the legume organisms. Magon and Dana (11) reported that

TABLE 3

Effect of different treatments on the production of nodules on soybeans

				TREA'	TREATMENT			
	Uninoculated	Bacterial	Bacterial infusion + soil	Bacterial infusion + glue	Bacterial infusion + soil + glue	Bacterial infusion + sugar	Bacterial infusion + sugar + soil	Bacterial infusion + sugar + soil + glue
Series 100—	Series 100—Planted immediately after inoculation (plants 25 days old)	diately after	rinoculation	(plants 25 d	ays old)			
Number of plants. Plants with nodules.	7 None				7 8	9 9	9	7
Size Number	None	Small Many	Medium Many	Medium Many	Medium Many	Large Many	Large Many	Large Many
Series 200-	Series 200-Planted 24 hours after inoculation, plants 25 days old)	ours after in	voculation" (p	lants 25 days	(plo)			
Number of plants	7 None	מו מו	ທທ	7		~ ~	9 25	2
Size	None	Small Many	Medium Many	Medium Many	Medium Many	Large	Large	Large Many
Series 300	Series 300—Planted 36 hours after inoculation (plants 23 days old)	ours after in	voculation (p	lants 23 day	(plo			
Number of plants. Plants with nodules. Nodule record:	4 None	4 4	4 4	w w	ທຸທ	4 4	8 8	4 4
Size	None	Small Many	Medium Many	Medium Many	Medium Many	Large Many	Large Few	Large Many

Series 400-Planted 48 hours after inoculation (plants 23 days old)

Number of plants.	4	2	4	4	w	9	4	7
	None	5	60	4	N	9	4	7
Size. Number.	None	Small	Medium Few	Medium	Medium	Large	Large	Large
Series 500—Planted 72 hours after inoculation (plants 20 days old)	Planted 72	ours after in	oculation (p	ants 20 days	(plo			
Number of plants.	7	25	20	4	9	7	9	9
	None	4	4	8	9	7	9	9
Size.	None	Small	Medium Medium	Medium	Medium	Large	Large	Large
Number	None	Many	Many	Many	Many	Many	Many	Many
Series 600	Planted 2	Series 600—Planted 2 weeks after inoculation (plants 24 days old)	oculation (p	ants 24 days	(plo:			
Number of plants.	7	7	9	7	Died	N	20	20
:	None	7	9	9	Died	S	Ŋ	N
Size number	None	Small	Medium	Medium	Died	Large	Large	Large
Number	None	Many	Many	Many	Died	Many	Many	Many
Series 700—Planted 1 month after inoculation (plants 26 days old)	Planted I m	onth after in	oculation (p	ants 26 days	(plo s			
Number of plants	4	1	9	ın	IO.	9	20	9
Plants with nodules	None	7	N	S	ю	9	LO .	S
Nodule record:	Mono	Cmall	Modium	Madium	Modium	Lordo	Largo	Targe
Number	None	Many	Many	Many	Many	Many	Many	Many
Series 900—Planted 2 months after inoculation (plants 30 days old)	Planted 2 m	onths after in	voculation (p	lants 30 day.	s old)			
Number of plants.	4	ıs	9	S	9	9	9	9
Plants with nodules	None	4	9	3	N)	9	8	N
Nodule record: Size.	None	Medium	Medium	Medium	Medium	Large	Large	Large
Number	None	Many	Many	Few	Many	Many	Many	Many

Effect of different treatments on the production of nodules on sweet clover

				TREATMENT	MENT			
	Uninoculated	Bacterial infusion	Bacterial infusion + soil	Bacterial infusion + glue	Bacterial infusion + soil + glue	Bacterial infusion + sugar	Bacterial infusion + sugar + soil	Bacterial infusion + sugar + soil + glue
Series 1000-	Series 1000—Planted immediately after inoculation (plants 45 days old)	ediately after	er inoculation	(plants 45 d	ays old)			
Number of plants.	. Aone		44	च च	ro ro		וא או	ro ro
Size	None None	Small Many	Medium Many	Medium Many	Medium Many	Large	Large Many	Large Many
Series 1010	Scries 1010—Planted 24 hours after inoculation (plants 44 days old)	ours after i	noculation (p	lants 44 day.	(plo s			
Number of plants	. None	9	4 4		מו מו	4 4	מו מו	7 7
Nodule record: Size Number	None None	Small	Medium Few	Medium Few	Medium Few	Large	Large Many	Large Many
	Series 1020-Planted 36 hours after inoculation (plants 44 days old)	ours after i	noculation (dants 44 day	(plo s			
Number of plants	. None	~ ~	99	2 2	- 1-	וה וה	9	9 4
Size Number	None None	Small	Medium Few	Medium Few	Medium Many	Medium Many	Large Many	Large Many

Series 2030—Planted 48 hours after inoculation (plants 43 days old)

Series 2030-Planted 48 hours after inoculation (plants 43 days old)

Number of plants	w	Ŋ	1	4	4	7	7	*
Plants with nodules	None	ıν	1	4	41	7	7	4
Size. Number.	None	Small	Medium	Medium Few	Medium	Large Many	Large Many	Large Few
Series 2040—Planted 72 hours after inoculation (Plants 42 days old)	Planted 72	hours after	inoculation (I	lants 42 day	(plo so			
Number of plants	4	4	9	N	3	7	9 .	1
Plants with nodules	None	4	9	N	m	7	9	1-
Size	None	Small	Medium	Medium	Medium	Large	Large	Large
Number	None	Few	Few	Few	Few	Many	Many	Many
Series 2050—Planted 2 weeks after inoculation (plants 28 days old)	Planted 2 1	veeks after i	noculation (p	lants 28 day.	(plo s			
Number of plants.	7	4	Died	7	9	7	1	7
	None	1	Died	1	9	7	7	7
Nodule record:								
Size	None	Small	Died	Small	Small	Medium	Medium	Medium
Number.	None	-	Died	Few	Few	Few	Many	Many
Series 2060—Planted 1 month after inoculation (plants 35 days old)	Planted I n	nonth after i	noculation (p	lants 35 day.	s old)			
Number of plants.	N	S	S	7	4	7	ın	7
Plants with nodules	None	N	4	2	4	1	10	1
Nodule record:								
Size	None	Small	Small	Small	Small	Large	Large	Large
Number	None	Few	Few	Few	Few	Many	Many	Many
Series 2070—Planted 2 months after inoculation (plants 52 days old)	lanted 2 m	ouths after 1	inoculation (p	ilants 52 day	(plo si			
Number of plants	2	65	7	1	7	-	2	3
Plants with nodules	None	3	7	1	7	-	2	8
Size	None	Small	Medium	Medium	Medium	Medium	Medium	Medium
Number	Mone	Fow	Few	Few	Few	Few	Fow	Many

TABLE 5
Effect of different treatments on the production of nodules on garden peas

				TREAT	TREATMENT			
	Uninoculated	Bacterial infusion	Bacterial infusion + soil	Bacterial infusion + glue	Bacterial infusion + soil + glue	Bacterial infusion + sugar	Bacterial infusion + sugar + soil	Bacterial infusion + sugar + soil + glue
Series 2080—Planted immediately after inoculation (plants 30 days old)	Planted imme	diately after	r inoculation	(plants 30 a	lays old)			
Number of plants	6 None	s 4	n n	4 4	7	9		
Size. Number.	None	Small Many	Medium Many	Medium Many	Medium Many	Large Many	Large Many	Large Many
Series 2090-	Series 2090—Planted 24 hours after inoculation (plants 30 days old)	tours after i	noculation (1	slants 30 day	(plo si			
Number of plants	7 None	NO 100	w w	א א	7	4 4	- 1	99
Size	None	Small Few	Medium Few	Medium	Medium Many	Large Many	Large Many	Large Many
Series 2300-	Series 2300—Pranted 36 hours after inoculation (plants 29 days old)	hours after 1	inoculation (blants 29 day	(plo si			
Number of plants. Plants with nodules. Nodula moods!	5 None	9 9	11	~ ~	7		1.1	- 1-
Size	None	Small Many	Medium Many	Medium Many	Medium Many	Large Many	Large Many	Large Many

Series 2310—Planted 48 hours after inoculation (plants 28 days old)

Series 2310-Planted 48 hours after inoculation (plants 28 days old)

Number of plants.	w	9	9	7	S	4	S	9
	None	9	9	7	10	4	, vo	9
Size	None None	Small Few	Medium Many	Medium Many	Medium Many	Large Many	Large Many	Large
Series 2320—Planted 72 hours after inoculation (plants 27 days old)	Planted 72	hours after	noculation (p	lants 27 day.	s old)			
Number of plants.	7	7	1	25	9	ı,	7	Died
Plants with nodules	None	7	-	Ŋ	9	Ŋ	7	Died
Size	None	Small	Medium	Medium	Medium	Large	Large	Died
Number	None	Few	Many	Few	Many	Many	Many	Died
Series 2330-	Planted 2	veeks after i	Series 2330—Planted 2 weeks after inoculation (plants 31 days old)	lants 31 days	(plo s			
Number of plants	9	9	7	ıo.	9	4	9	4
Plants with nodules	None	1	4	85	S	4	4	4
Size	None	Small	Medium	Medium	Medium	Large	Large	Large
er	None	Few	Few	Few	Few	Few	Many	Many
Series 2340—Planted 1 month after inoculation (plants 31 days old)	Planted 1 n	nonth after i	noculation (p	lants 31 days	(plo s			
Number of plants	7	7	9	7	9	1	7	7
Plants with nodules	None	7	9	7	9	2	7	-
Size.	None	Small	Medium	Medium	Medium	Large	Large	Large
Number	None	Many	Many	Many	Few	Many	Many	Many
Series 2350—Planted 2 months after inoculation (plants 28 days old)	Planted 2 n	conths after	inoculation (slants 28 day	(plo so			
Number of plants	4	8	10	Ŋ	9	25	9	9
Plants with nodules	None	3	ທ	Ŋ	9	Ŋ	9	9
Size	None	Small	Medium	Medium	Medium	Large	Large	Large
Number	None	Frw	Many	Many	Many	Many	Many	Many

the use of glue with the bacterial infusion was injurious because it was heavily infected with different kinds of bacteria. The fact that the nodule production in the treatment containing bacterial infusion and glue was superior to the untreated bacterial infusion in this experiment, was probably due to the fact that the seeds were twice mixed in applying both the glue and the bacterial infusion. Fellers (5) reported that the use of glue with the bacterial infusion gave no benefit, but, rather, was injurious to the legume organisms, as it contained different kinds of bacteria.

TABLE 6
Additions made to bacterial infusion in experiment 2

LOT	TREATMENT
1	5 cc. bacterial infusion
2	5 cc. bacterial infusion + 7 gm. cane sugar
3	5 cc. bacterial infusion + 30 gm. air-dried brown silt loam
4	7 gm. cane sugar dissolved in 5 cc. bacterial infusion
5	30 gm. air-dried brown silt loam + 7 gm. cane sugar dissolved in 5 cc. bacterial infusion
6	30 gm. air-dried brown silt loam mixed with 5 cc. bacterial infusion

TABLE 7
Series and intervals of planting

TABLE 8

	-	
INTERVALS OF PLANTING	GARDE	N PEAS
ANTARIN FRANCI VA A MICHA ANTO	Series A-wrinkled	Series B-smooth
5 days after inoculation	2,360	2,400
15 days after inoculation	2,370	2,410
20 days after inoculation	2,380	2,420
30 days after inoculation	2,390	2,430

Experiment 2—The effect upon nodule production of sugar and soil in different combinations with the infusion

The purpose of this experiment was to determine whether sugar and soil, with the infusion in different combinations, would stimulate nodule production sufficiently to be of real value.

The experiment was divided into parts, A and B. In part A, wrinkled garden peas were used, and in part B, smooth garden peas. The same procedure was used for each experiment. Table 6 shows the treatments applied.

Each lot consisted of 1 quart of seed. In all cases, the mixtures that were added were applied to the seed and the seed was thoroughly mixed. It will be seen from table 6 that, in some cases, the sugar and soil were mixed with the infusion before the mixture was applied to the seed, whereas in other cases, the infusion was applied to the seed, and the soil and sugar were applied afterwards.

In lots 4, 5, and 6, the added materials were dissolved in the bacterial infusion

TABLE 8

Effect of different treatments on the production of nodules on wrinkled garden peas

				TREATMENT			
	Uninoculated	Bacterial	Bacterial infusion + sugar	Bacterial infusion + sugar + soil	Sugar + infusion mixed	Sugar + soil +	Soil + infusion mixed
Series	2360-Planted	5 days after	Series 2360—Planted 5 days after inoculation (plants 30 days old)	uts 30 days old	()		
Number of plants	4	9	9	9	9	7	7
Plants with nodules	None	ທ	9	S	9	_	4
Size. Number.	None None	Small Few	Large	Large Many	Large Many	Large Many	Large Many
Series	2370-Planted	15 days after	Series 2370-Planted 15 days after inoculation (plants 40 days old)	nts 40 days old	()		
Number of plants	9	9	s	4	3	9	20
Plants with nodules	None	S	2	4	3	9	ın
Size	None	Small	Large	Large	Small	Large	Medium
Number	None	Few	Many	Many	Few	Many	Many
Series	2380-Planted	20 days after	Series 2380-Planted 20 days after inocubation (plants 30 days old)	nts 30 days ola	(1)		
Number of plants	4	ın	4	ıo	9	3	4
Plants with nodules	None	N	4	ı,	9	3	4
Nodule record:	,					-	
Size. Number.	None	Few	Large	Large	Large	Large	Large
Series	2390-Planted	30 days after	Series 2390—Planted 30 days after inoculation (plants 29 days old)	nts 29 days ol			
Number of plants	9	4	9	9	r.	7	Died
Plants with nodules.	None	4	3	9	S	7	Died
Nodule record:	None	Small	Larce	Laron	Large	Large	Died
Number	None	Few	Many	Many	Many	Many	Died

TABLE 9
Effect of different treatments on the production of nodules on smooth Alaska peas

				TREATMENT			
	Uninoculated	Bacterial	Bacterial infusion + sugar	Bacterial infusion + sugar + soil	Sugar + infusion mixed	Sugar + soil +	Soil + infusion mixed
Series	2400-Planted	5 days after	Series 2400—Planted 5 days after inoculation (plants 19 days old)	uts 19 days of	(p		
Number of plants	o None	4 11	4 2	ונו מו	6 6	4 4	2 2
: :		Small	Medium Many	Medium	Medium	Large Many	Small
Series	2410-Planted	15 days after	Series 2410—Planted 15 days after inoculation (plants 31 days old)	nts 31 days o	(p)		
Number of plants.	4	80	60	8	4	4	4
Plants with nodules	None	2	8	ю	4	4	4
Size	None	Small	Large	Large	Large	Large	Large
Number	None	Few	Many	Many	Many	Many	Many
Series 24	420-Planted 2	O days after	Series 2420—Planted 20 days after inoculation (plants 24 days old)	mts 24 days	old)		
Number of plants.	10	4	9	ro.	4	2	S
Plants with nodules	None	4	9	Ŋ	4	ro.	w
Size.	None	Medium	Large	Large	Large	Large	Large
Number	None	Many	Many	Many	Many	Many	Many
Series	2430-Planted	30 days after	Series 2430—Planted 30 days after inoculation (plants 21 days old)	ints 21 days o	(p)		
Number of plants	ıs	8	20	S	3	9	3
Plants with nodules	None	8	ro	N	3	9	8
Size		Small	Large	Large	Large	Large	Large
Number	None	Many	Many	Many	Many	Many	Many

before being applied to the seed. After the applications were made, the seeds of all lots were dried in enameled pans and then transferred to cloth seed bags and stored in the laboratory locker.

The results of experiment 2, part A, are reported in table 8. In the untreated bacterial infusion, the nodule production was not constant in all the plantings. The number of nodules produced at 5-days planting was about one-third as great as at 15-days. The 20-days planting produced one-half as many nodules as the 30-days. Numerous nodules were produced on each plant in all the treatments (lots 2, 3, 4, 5, 6), and the nodule production was constant for a given treatment, in all the different plantings.

TABLE 10

Calcium carbonate, tricalcium phosphate, and sugar applied with bacterial infusion in experiment 3

NUMBER	TREATMENT
1	10 per cent cane sugar + brown silt loam
2	1 per cent cane sugar + brown silt loam
3	0.1 per cent cane sugar + brown silt loam
4	0.01 per cent cane sugar + brown silt loam
5	1 per cent calcium carbonate + gray silt loam on tight clay
6	0.1 per cent calcium carbonate + gray silt loam
7	0.01 per cent calcium carbonate + gray silt loam
8	1 per cent tricalcium phosphate + brown silt loam
9	0.1 per cent tricalcium phosphate + brown silt loum
10	0.01 per cent tricalcium phosphate + brown silt loam

TABLE 11
Intervals of planting

SERIES-PEAS	TIME OF STORAGE OF SOIL BEFORE INOCULATING SEEDS	TIME OF STORAGE OF SEEDS AFTER INOCULATION BUT BEFORE PLANTING
2,480	Inoculated immediately	Planted immediately
2,500	Inoculated immediately	10 days
2,520	Inoculated immediately	20 days
2,540	Inoculated immediately	30 days
2,560	41 days	Planted immediately
2,580	107 days	Planted immediately

The results of part B are reported in table 9. In the lot receiving bacterial infusion alone, the nodules produced were numerous at the 5-, 20-, and 30-days planting, but decreased to one-third as many at 15-days. All the treatments (lots 2, 3, 4, 5, 6) resulted in numerous and constant nodule production in all of the plantings.

The fluctuation in nodule production which was observed in the lot receiving bacterial infusion alone was not found in lots receiving the other treatments. It was noticed that with sugar either applied on moist inoculated seeds or first

The effect of calcium carbonate, tricalcium phosphate, and sugar in various amounts in soil on the duration of the life of nodule bacteria after being applied to the seed TABLE 12

	SERIES	2480-PL APTER I (PLANTS	SERIES 2480—PLANTED IMMEDIATELY AFFER INOCULATION (PLANTS 29 DAYS OLD)	DIATELY	SERIES	2500-PL INO (PLANTS	SERIES 2500—PLANTED 10 DAYS AFTER (PLANTS 26 DAYS OLD)	ES APTER
TREATMENT	Number	Plants	Nodule record		Number Plants	Plants	Nodule record	record
	plants	nodules	Size	Number	plants	nodules	Size	Number
Uninoculated	2	None	None	None	NO.	None	None	None
Bacterial infusion	4	4	Small	Few	3	8	Medium	Many
Bacterial infusion + soil (B. S. L.)*	5	S	Medium	Many	3	3	Medium	Many
Bacterial infusion + soil (G. S. L.)†	5	S	Medium	Many	7	2	Medium	Many
Bacterial infusion + soil + 10 per cent sugar	. 2	2	Medium	Few	4	4	Large	Few
Bacterial infusion + soil + 1 per cent sugar		3	Medium	Many	2	2	Large	Many
Bacterial infusion + soil + 0.1 per cent sugar	9	9	Medium	Many	4	4	Large	Many
Bacterial infusion + soil + 0.01 per cent sugar	20	S	Large	Many	4	4	Large	Many
Bacterial infusion + soil + 1 per cent CaCO ₃	, N	2	Small	Many	S	Ŋ	Large	Man
Bacterial infusion + soil + 0.1 per cent CaCO ₃	9	S	Medium	Many	3	3	Large	Man
Bacterial infusion + soil + 0.01 per cent CaCO ₃		3	Medium	Few	4	4	Large	Many
Bacterial infusion + soil + 1 per cent Ca ₃ (PO ₄) ₂	4	4	Large	Many	S	2	Large	Many
Bacterial infusion + soil + 0.1 per cent Ca ₃ (PO ₄) ₂	7	1	Large	Many	3	3	Large	Many
Bacterial infusion + soil + 0.01 per cent Ca ₃ (PO ₄) ₂	9	9	Large	Many	r)	S	Large	Many
Bacterial infusion + soil + 0.1 per cent each Ca ₂ (PO ₄) ₂ + sugar + CaCO ₃	4	4	Large	Many	7	2	Medium	Many
Bacterial infusion + soil (B. S. L.)* + 0.1 per cent CaCO ₃	2	S	Large	Many	Died	Died	Died	Died

	SERIES	2520—PL INO (PLANTS	SERIES 2520—PLANTED 20 DAYS AFTER (PLANTS 22 DAYS OLD)	rs Arter	SERIES	2540—PL INO (PLANTS	SERIES 2540—PLANTED 30 DAYS AFFER INOCULATION (FLANTS 22 DAYS OLD)	XS AFTER
Uninoculated	N	None		None	8	None		None
Bacterial infusion.	4	None		None	2	2	Small	Few
Bacterial infusion + soil (B. S. L.)*	3	2	Small	Few	S	None		None
Bacterial infusion + soil (G. S. L.)†	3	None		None	S	None	:	None
Bacterial infusion + soil + 10 per cent sugar	4	None		None	4	None	:	None
Bacterial infusion + soil + 1 per cent sugar	4	None		None	Ŋ	None		None
Bacterial infusion + soil + 0.1 per cent sugar	3	None		None	S	None		None
Bacterial infusion + soil + 0.01 per cent sugar	3	None		None	4	None	:	None
Bacterial infusion + soil + 1 per cent CaCO ₃	4	1	Small	3	9	None		None
Bacterial infusion + soil + 0.1 per cent CaCOs	25	None		None	4	None		None
Bacterial infusion + soil + 0.01 per cent CaCO ₃	S	None		None	3	None		None
Bacterial infusion + soil + 1 per cent Ca ₃ (PO ₄) ₃	S	None		None	S	None		None
Bacterial infusion + soil 0.1 per cent Cas(POs)2	4	None		None	4	None	:	None
Bacterial infusion + soil + 0.01 per cent Ca ₂ (PO ₄) ₃	4	None		None	S	None		None
Bacterial infusion + soil + 1 per cent each Ca ₂ (PO ₄) ₂ + sugar + CaCO ₃	3	None		None	4	1	Small	-
Bacterial infusion + soil (B. S. L.)* + 0.1 per cent CaCO ₃	Ŋ	None		None	Ŋ	None		None

* Brown silt loam.

 \dagger Gray silt loam. Sugar and Ca,(PO4)2 used on brown silt loam, and Ca,CO3 used on gray silt loam.

dissolved in infusion, the nodules produced were larger and more uniform in size. Whether this effect was due to the stimulative action of sugar or to other factors was not determined. Soil alone showed a slight benefit but not so much as sugar.

A comparison of the data reported in tables 8 and 9 reveals no marked difference between smooth and wrinkled peas with respect to nodule production. The relative smoothness of the seed coat may therefore be considered a negligible factor.

Experiment 3—The effect of varying amounts of calcium carbonate, tricalcium phosphate, and sugar in soil on the duration of the life of nodule bacteria in the soil and after being applied to the seed

Two types of soil were used, brown silt loam having a lime requirement of 300 pounds, and gray silt loam on tight clay having a lime requirement of 1300 pounds as determined by the Hopkins' method. The brown silt loam was used with sugar and tricalcium phosphate and also with calcium carbonate. The gray silt loam was used only with calcium carbonate. Pure cultures of garden pea bacteria were tested in this experiment.

One pound of soil was the basis for each preparation. The inoculation was on the basis of 5 cc. of infusion of pea bacteria for each pound of soil. Each mixture was prepared and thoroughly mixed before the infusion was added. The amount for each substance used was on the percentage basis. The soils contained 20 per cent moisture when mixed. After preparation, the various treatments were placed in pint glass jars provided with caps and were stored on the side shelf in the laboratory.

Two grams of the mixture was used to inoculate 1 ounce of sterilized pea seeds and a few cubic centimeters of sterile water was added while inoculating in order to secure thorough distribution of the organisms. The inoculated seeds were placed in glass tumblers and stored in a drawer in the laboratory.

The intervals of planting are shown in table 11.

The data reported in table 12 show that the seeds planted immediately after inoculation as well as those stored 10 days after being inoculated, in all cases, produced numerous nodules. The untreated bacterial infusion showed greater fluctuations in the number of nodules than any of the other treatments. A very striking result was obtained after the seed had been stored 20 days before planting. No nodules were produced from this planting except with the treatment containing the bacterial infusion and brown silt loam, where a few small nodules were found, and also in the treatment containing the bacterial infusion and 1 per cent calcium carbonate, where 3 nodules were found on one plant. This, however, is looked upon as accidental. Storing the inoculated seeds for 30 days before planting resulted in no nodule production in any of the treatments except in the untreated bacterial infusion, which produced a few small nodules, and also in the treatment containing bacterial infusion, soil, and $\frac{1}{10}$ per cent each of tricalcium phosphate, calcium carbonate, and sugar, where 1 nodule was found on one plant.

It is quite apparent from the above statements that the bacteria in the infusion applied to these soils in conjunction with sugar, limestone, and phosphate, died out very rapidly, and were nearly extinct in 20 days. It should be remembered that these mixtures were made with soil, portions of which were

TABLE 13

The effect of varying amounts of calcium carbonate, tricalcium phosphate, and sugar upon the duration of life of nodule bacteria in moist stored soil

	NUMBER	PLANTS	NODULE	RECORD
TREATMENT	OF PLANTS	WITH	Size	Number
Series 2560—Soil stored 41 days before inoculating seed. days old)	s for imn	nediate 1	blanting (p	lants 2
Uninoculated	3	None		None
Bacterial infusion + soil (B. S. L.)*	5	5	Medium	Many
Bacterial infusion + soil (G. S. L.)†	4	4	Medium	Many
Bacterial infusion + soil + 10 per cent sugar	4	None		None
Bacterial infusion + soil + 1 per cent sugar	4	None		None
Bacterial infusion + soil + 0.1 per cent sugar	4	4	Medium	Many
Bacterial infusion + soil + 0.01 per cent sugar	4	4	Medium	Many
Bacterial infusion + soil + 1 per cent CaCO ₃	5	5	Medium	Many
Bacterial infusion + soil + 0.1 per cent CaCO ₃	2	2	Medium	Few
Bacterial infusion + soil + 0.01 per cent CaCO ₃	Died	Died	Died	Died
Bacterial infusion + soil + 1 per cent Ca ₃ (PO ₄) ₂	5	5	Medium	Many
Bacterial infusion + soil + 0.1 per cent Ca ₃ (PO ₄) ₂	3	3	Medium	Many
Bacterial infusion + soil + 0.01 per cent Ca ₃ (PO ₄) ₂	4	4	Medium	Many
Bacterial infusion + soil + 0.01 per cent Ca ₃ (PO ₄) ₂ +				
sugar + CaCO ₃	3	3	Medium	Many
Bacterial infusion + soil + 0.1 per cent CaCO ₃	5	5	Medium	Many
Series 2580—Soil stored 107 days before inoculating seed: days old)	s for im	mediate	planting (p	lants 30
Jninoculated	5	None		None
Bacterial infusion + soil (B. S. L.)*	5	5	Large	Many
Bacterial infusion + soil (G. S. L.)†	5	5	Large	Many
Bacterial infusion + soil + 0.1 per cent sugar	4	4	Large	Many
Bacterial infusion + soil + 0.01 per cent sugar	3	3	Large	Many
Bacterial infusion + soil + 1 per cent CaCO ₃	5	5	Medium	Many
Bacterial infusion + soil + 0.01 per cent CaCO ₃	6	6	Medium	Many
	5	5	Medium	Many
Bacterial infusion + soil + 1 per cent Ca ₃ (PO ₄) ₂ .				

^{*} Brown silt loam.

used immediately for inoculating the seed, after which the seed remained in storage for the time stated. The mixtures of soil with limestone, phosphate, and sugar were kept moist and at the end of 41 days and 107 days, respectively, portions of these moist mixtures were applied, as in the first part of this experiment, to sterile seeds, and the seeds were planted immediately.

[†] Gray silt loam.

It may be pointed out that the same mixtures, when used immediately and allowed to dry out in a very short time, would be expected to perform quite differently from when kept moist for a number of months before being applied to the seed. In the first case, there would be little opportunity for the limestone to correct acid soil conditions and for the sugar to supply a source of energy, whereas in the second case, ample opportunity exists for these changes. The contact of the organisms with the acid soil in the first case was greater than the contact with the corrective materials. Because of the above conditions, it was anticipated that entirely different results would be obtained in the two cases.

The results presented in table 13 indicate that soils stored moist for 41 days with the above named treatments, produced infection on peas in all cases except 3, namely, the uninoculated soils and those receiving respectively 10 per cent and $\frac{1}{10}$ per cent sugar. When the period of storage was 107 days, nodules were produced in all cases except with the uninoculated checks.

Experiment 4—The effect of different containers for storing inoculated seed upon the life of legume organisms

The seeds used for this experiment were inoculated seeds with the same treatments as in experiment 1. The inoculated and treated seeds of each of the different legumes were mixed thoroughly and divided into two portions. One portion of 2 quarts was stored in a 2-quart Mason glass jar, and the other portion containing 10 quarts was stored in a seed bag. The jars were stored in the laboratory locker, and the bags in the laboratory store room. Humidity and temperature conditions were approximately the same in both places.

After 5-months storage, the variously treated seeds were planted in pint jars of sterilized quartz sand. Seven seeds were planted in each jar and 5 jars were used for each lot of seeds. All the plants were allowed to grow for 30 days in order to insure the maximum nodule production.

All plants grew normally up to 20 days, but from that time on, the plants from seeds stored in glass jars became yellow and stopped growing, whereas the plants from seeds stored in bags were green and continued to grow. Examination showed that nodules were absent on the roots of all the plants from seeds stored in glass containers, whereas the plants from seeds stored in bags developed several nodules. The results were the same for the three legumes used, soybeans, sweet clover, and garden peas.

PART II.—THE ENDURANCE OF NODULE BACTERIA IN THE PRESENCE OF OTHER ORGANISMS, AND IN THE PRESENCE OF EACH OTHER

Experiment 5—The endurance of pea bacteria in the presence of B. radiobacter

One cubic centimeter each of pea bacteria infusion and B. radiobacter infusion was transferred into each of 2 bottles of mannite agar media and 2

bottles of cane sugar media. After incubation at 26°C. for 10, 20, and 30 days, portions were tested on potato, in milk, and for inoculation of peas.

Test on milk. One loopful of infusion from each bottle was transferred into a test tube of sterile milk. The tubes were incubated at 26°C. and examinations were made at different periods.

Test on potato slant. One loopful of infusion from each bottle was transferred to potato slant prepared in test tubes. These tubes were incubated at 26°C, and examinations were made.

Test on inoculation. One cubic centimeter of infusion from each bottle was used to inoculate $\frac{1}{2}$ ounce of sterilized pea seeds. Five inoculated pea seeds were immediately planted in jars from each $1\frac{1}{2}$ -ounce lot of seed. Four replicates from each lot were thus planted.

Experiment 6—The endurance of cowpeas, soybean, and sweet clover organisms in the presence of B. radiobacter

Three separate mixtures were prepared of *B. radiobacter*, with cowpea, soybean, and sweet clover bacteria respectively, 1-cc. portions of the respective infusions being used in each case. These were incubated, as in experiment 5, on both mannite and cane sugar agar at 26°C. and tested at 10-, 20-, and 30-day intervals in milk, on potato slants, and for ability to produce nodules.

Experiment 7—The endurance of pea, cowpea, soybean and sweet clover bacteria in the presence of Azotobacter chroococcum

Garden pea, cowpea, soybean, and sweet clover bacteria were grown in the presence of *Azotobacter chroococcum* as in experiment 6 except that only mannite agar was used, and the tests on potato slant were omitted.

Experiment 8—The endurance of pea bacteria in the presence of B. prodigiosus, B. capsulatus, B. subtilis, B. mesentericus, pink yeast, and molds

A loopful of an infusion of pea bacteria was mixed with a loopful of an infusion of each of B. prodigiosus, B. capsulatus, B. subtilis, B. mesentericus, pink yeast, and molds in separate tubes of sterile milk. The tubes were incubated for 14 days at 26°C., and after the results of the growth in milk were recorded, tests for inoculation were conducted. This experiment was carried out in duplicate tubes.

In all the bottles where the legume organisms of different kinds were grown with *B. radiobacter*, the growth appearing on the agar was typical of *B. radiobacter*. This was due to the fact that *B. radiobacter* grew faster than any legume organisms used. It would seem that the legume organisms would soon perish, either for lack of food or oxygen. Although they might have been deprived of food and oxygen, their viability was very little, or not at all, affected as measured by nodule production. The legume organisms may have changed



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Test on milk. One loopful of infusion from each bottle was transferred into a test tube of sterile milk. The tubes were incubated at 26°C. and examinations were made at different periods.

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TABLE 14

The grouth of coupea, soybean, and sweet clover organisms with B. radiobacter on mannite agar media
Test on potato

NUMBER OF TUBES	ORGANISMS	INCUBATION PERIOD ON MANNITE AGAR	DESCRIPTION OF GROWTH ON POTATO AFTER SIX DAYS
Managaria Professional Professi		days	
6	Uninoculated		Potato unchanged
4	Cowpea bacteria + B. radiobacter	10	Potato turned gray
4	Cowpea bacteria + B. radiobacter	20	Potato turned gray
4	Cowpea bacteria + B. radiobacter	30	Potato turned gray
4	Soybean bacteria + B. radiobacter	10	Potato turned gray
4	Soybean bacteria $+ B. radiobacter$	20	Potato turned gray
4	Soybean bacteria + B. radiobacter	30	Potato turned gray
4	Sweet clover bacteria + B. radiobacter	10	Potato turned gray
**	Sweet clover bacteria $+ B$. radiobacter	20	Potato turned gray
4	Sweet clover bacteria $+ B$. radiobacter	30	Potato turned gray

	I est	I est in misk		
NUMBER OF	ORGANISHS	INCUBATION PERIOD ON	DESCRIPT	DESCRIPTION AFTER
TUBES		MANNITE AGAR	4 days	14 days
		days		
6	Uninoculated		Milk unchanged	Milk unchanged
4	Cowpea bacteria $+ B. radiobacter$	10	Clear serum on top	Milk turned brown
41	Cowpea bacteria + B. radiobacter	20	Clear serum on top	Milk turned brown
4	Cowpea bacteria + B. radiobacter	30	Clear serum on top	Milk turned brown
4	Soybean bacteria + B. radiobacter	10	Clear serum on top	Milk turned brown
4	Soybean bacteria + B. radiobacter	20	Clear serum on top	Milk turned brown
4	Soybean bacteria + B. radiobacter	30	Clear serum on top	Milk turned brown
4	Sweet clover bacteria + B. radiobacter	10	Clear serum on top	Milk turned brown
4	Sweet clover bacteria + B. radiobacter	20	Clear serum on top	Milk turned brown
4	Sweet clover bacteria $+ B.$ radiobacter	30	Clear serum on top	Milk turned brown

Test for inoculation (plants 30 days old)

NUMBER OF JARS	ORGANISMS	INCUBATION PERIOD ON MANNITE AGAR	NUMBER OF PLANTS	PLANTS WITH NODULES
		days		
18	Uninoculated		11	None
4	Cowpea bacteria $+ B.$ radiobacter	10	14	14
4	Cowpea bacteria $+ B. radiobacter$	20	16	16
4	Cowpea bacteria + B. radiobacter	30	16	16
4	Soybean bacteria + B. radiobacter	10	11	11
4	Soybean bacteria $+ B. radobacter$	20	14	14
4	Soybean bacteria + B. radiobacter	30	14	14
4	Sweet clover bacteria $+ B. radiobacter$	10	22	22
4	Sweet clover bacteria + B. radiobacter	20	20	20
4	Sweet clover bacteria $+ B$. radiobacter	30	13	13

The growth of pea, cowpea, soybean, and sweet clover bacteria with Azotobacter chroococcum on mannite agar media Test in milk TABLE 15

NUMBER	SWEINAGO	INCUBATION		DESCRIPTION AFTER
OF TUBES		MANNITE	8 days	13 days
		days		
_	Uninoculated		Milk unchanged	Milk unchanged
_	Pea bacteria + Azotobacter	10	Clear serum on top	Milk turned watery (pea)
	Pea bacteria + Azotobacter	20	Clear serum on top	Milk turned watery (pea)
_	Pea bacteria + Azotobacter	30	Clear serum on top	Milk turned watery (pea)
	Cowpea bacteria + Azotobacter	10	Clear serum on top	Brown precipitate (Azotobacter)
	Cowpea bacteria + Azotobacter	20	Clear serum on top	Brown precipitate (Azotobacter)
	Cowpea bacteria + Azotobacter	30	Clear serum on top	Brown precipitate (Azotobacter)
	Soybean bacteria + Azotobacter	10	Clear serum on top	Brown precipitate (Azotobacter)
	Soybean bacteria + Azotobacter	20	Clear serum on top	Brown precipitate (Azotobacter)
	Soybean bacteria + Azotobacter	30	Clear serum on top	Brown precipitate (Azotobacter)
	Sweet clover bacteria + Azotobacter	10	Clear serum on top	Milk turned watery (sweet clover)
	Sweet clover bacteria + Azotobacter	20	Clear serum on top	Milk turned watery (sweet clover)

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NUMBER OF JARS	ORGANIBMS	INCUBATION PERIOD ON MANNITE	NUMBER OF PLANTS	PLANTS WITH NODULES
		days		
9	Uninoculated		25	None
2	Pea bacteria + Azotobacter	10	_	2
2	Pea bacteria + Azotobacter	20	00	00
2	Pea bacteria + Azotobacter	30	00	00
2	Cowpea bacteria + Azotobacter	10	00	00
2	Cowpea bacteria + Azotobacter	20	10	10
2	Cowpea bacteria + Azotobacter	30	7	7
2	Soybean bacteria + Azotobacter	10	11	11
2	Soybean bacteria + Azotobacter	20	7	1
2	Soybean bacteria + Azotobacter	30	1	1
2	Sweet clover bacteria + Azotobacter	10	1	1
2	Sweet clover bacteria + Azotobacter	20	7	7
2	Sweet clover bacteria + Azotobacter	30	00	00

their stage of existence, or may have been living as well as B. radiobacter. There may be a mutual relationship between the legume organisms and B. radiobacter but this has not been determined. Löhnis and Hansen (9) stated, "Bacillus radiobacter seems to be regularly present in root nodules of leguminous plants, stimulating development and activity." They also reported that B. radiobacter and B. radicicola are closely related to each other as shown in the morphology of the cell.

Whiting has further studied the question of the beneficial influence of B. radiobacter on cowpeas, soybeans, and other legumes, but has not found tangible evidence that B. radiobacter is desirable. On the contrary, he has found this organism destructive to nodule production in certain mixed cultures.

The fact that tests on potato and in milk showed the presence of B. radiobacter, does not necessarily mean that the legume organisms were dead or absent.

In the case where Azotobacter chroococcum was used instead of B. radiobacter, the infective power of different legume organisms used was not hindered, as is shown by inoculation test.

2 8

Sweet clover bacteria + Azotobacter Sweet clover bacteria + Azotobacter

2 2

Azotobacter

The survival of legume organisms in the presence of certain contaminating organisms of course depends upon many factors, particularly when grown on artificial media. The time factor is one very important consideration. In these experiments, transferring did not enter in, and this, of course, might greatly alter the results.

SUMMARY

In experiment 1 where inoculated seeds were variously treated with soil, glue, and sugar, applied alone and in combination, some legume organisms remained variable and nodule production occurred after 60-days storage in small seed bags under ordinary conditions.

In the treatments where sugar was used, applied either alone or with glue or soil, the nodules developed were uniformly large, and evenly distributed over the root system. Soil and glue did not show any particular advantages over the untreated bacterial infusion.

The irregularity in nodule production as shown in experiment 1 at different times of planting was thought to be due to the mechanical effect of mixing at the time of inoculation. The most pronounced irregularity occurred with sweet clover, probably because of the small size of the seed with consequent greater surface to cover.

The results of experiment 2 showed that the sugar treatment, either dissolved in bacterial infusion and then applied to the seeds, or applied on inoculated seeds, gave better results in nodule production than soil prepared in the same manner. The use of soil with sugar gave no better results than sugar

No difference in nodule production was noticed between treatments dissolved in bacterial infusion previous to its application on sterile seeds and treatments applied directly on inoculated seeds before mixing.

No significant difference in nodule production was noticed between sugar, tricalcium phosphate, and calcium carbonate in different amounts with infected soil when used for inoculation.

Soils containing 10 per cent sugar developed acidity which was entirely unfavorable to the life of *B. radicicola*. This organism remained alive in moist soils after 107-days storage in pint glass jars with various treatments.

Cloth seed bags for storing inoculated seeds are far superior to glass containers for maintaining the life of the legume organisms.

B. radiobacter and Azotobacter chroococcum showed no harmful effects upon the life and infecting power of the nodule bacteria under the conditions established and during the time tested.

Soybean, sweet clover, cowpea, and garden pea bacteria, when grown together, showed no harmful effect upon each other.

The activity of pea bacteria when grown in milk was not impaired by the presence of any one of the organisms tested; namely, B. prodigiosus, B. capsulatus, B. subtilis, B. mesentericus, pink yeast, and molds.

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A NEW SOIL CORE SAMPLER

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The proper sampling of a soil is no simple undertaking. Both care and experience are required for best results. For many purposes, the old methods of securing representative samples with the auger or spade are satisfactory because the physical disturbance to which the soil is subjected does not alter its chemical composition. When the detailed physical properties of the natural soil are to be studied, however, a modified method of sampling is needed.

Certain colloid investigations at the Missouri Experiment Station made desirable some very careful studies on the rates of percolation of water through a heavy subsoil when treated with various salts. It was, therefore, imperative that the structure of the soil should remain undisturbed, In order to accomplish this it was necessary to develop a sampler that would take an undisturbed core of soil with the desired dimensions. This necessity encouraged the development of the sampler described in this paper.¹

The accompanying detailed drawing shows the plan of the sampler, which consists essentially of two cyclinders, one within the other, the outer one being furnished with cutting knives. A is the top view of the outer cylinder showing steel head and three brass rollers. This cylinder head has a threaded hole $2\frac{1}{8}$ inches in diameter for the insertion of handle H. These three brass rollers spaced at equal distances around the cylinder are 1 inch in diameter and $\frac{1}{18}$ inch thick. They roll on rim D which fits on cylinder F when in operation. These rollers are secured to the wall of cylinder C. B is a side view of the same cylinder head, showing raised center for the support of handle H. This cut also shows a groove for the rollers, which have already been mentioned. The cylinder head B fits into cylinder C where it is secured by four screws, two of which are shown in the drawing.

Cylinder C is heated so it will go over the lower portion of head B and shrink

¹ In Bulletin 94 of the Iowa Agricultural Experiment Station, W. H. Stevenson describes a sampler devised and used at that station. It is to this piece of work that the writer is indebted for many valuable suggestions used in the development of this sampler. Acknowledgment is made to Dr. R. Bradfield and to Dr. W. A. Albrecht of this station for their assistance in the development of this sampler. Much credit also must be given to Mr. Gus. Tornsjo, chief mechanician of the university, to whose skill the working out of the mechanical details was largely due.

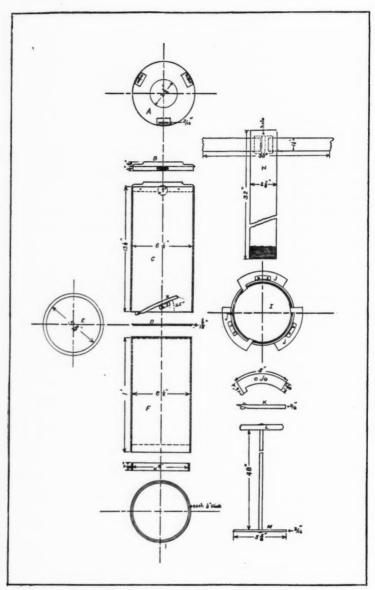


FIG. 1. PLAN OF THE NEW SOIL CORE SAMPLER

tightly in place. This prevents the cylinder from becoming distorted when force is applied to rotate the sampler in hard soil. Cylinder C is brass, the wall is $\frac{1}{8}$ inch thick and $13\frac{1}{8}$ inches long. The inside diameter is $6\frac{1}{2}$ inches. The knives, one of which is shown as K, are fastened to the outer wall of this cylinder at an angle of 25° as shown in the drawing, and are removable. The rim D is steel; it is $\frac{7}{16}$ inch thick and $\frac{1}{16}$ inch wide. This rim has a shoulder which fits on top of cylinder F; the lower portion extending into the top of the cylinder as shown. This rim is removable and furnishes a track upon which the rollers in cylinder C operate. E is the top view of rim D. Cylinder F is also made of brass, and has a wall $\frac{1}{8}$ inch thick. It fits just inside cylinder C. Both cylinders are bored true. At the lower end of cylinder F there is a stationary band $N, \frac{7}{8}$ inch wide and $\frac{1}{8}$ inch thick. This band is made of brass and is not removable. G is an end view of cylinder F with the included band. H is the handle complete. These parts are made from gas pipes with dimensions as given on the drawing. The threaded end of this handle screws into the cylinder head A. The cross handles are connected in the center as indicated. The plunder rod extends through this hole.

I is an end view of the sampler showing the knives, J, in place. The innertips of these knives are bronze, are soldered on the steel knives, and extend just inside of the inner rim of the band N. J shows a knife as it appears from the bottom view when detached. K is the same knive showing the edge view. Plunger M has a steel disc $5\frac{5}{8}$ inches in diameter. It fits in cylinder F with the handle projecting through handle H of the sampler. The plunger handle L is removable. It has a square hole through the center which fits the square end of the plunger rod. This plunger rod is used to push cylinder F out of cylinder C, then it is inserted into the lower end of cylinder F to remove the core of soil.

When taking a core of soil with this sampler, all grass and trash must be removed from the surface of the soil, for if such material is not removed it will hang on the knives and as a result, the upper part of the core will be disturbed.

Considerable care is required to start the sampler, as it may slip about before taking hold. To prevent this, a frame may be built to hold it steady. Such a frame is shown in the accompanying cut. No definite type of frame is needed, but the sampler must be held steady. The frame holds the sampler true while it is being operated so that the core will not be broken or disturbed. The rotation of the sampler causes the knives to cut away a strip of soil $1\frac{1}{2}$ inches wide. This rotation is continued until the desired depth is reached—up to 12 inches—after which the core is broken off below the cylinder by pulling the handle to one side.

The sampler is then removed from the soil, placed against the frame as shown in plate 1, figure 1, and the knives are taken off. The inner cylinder is taken out, rim D removed and the plunger is inserted in the other end of cylinder F to push out the sample. The core of soil continues to rest on the plunger until it is carefully wrapped with screen wire. (Plate 1, Fig. 2.) The wrapped core

is then made air-tight by dipping it in a can of hot paraffine. It is allowed to cool and is redipped until the desired thickness of paraffine is secured. This is best done in the field.

This sampler has been used with satisfaction on both surface soils and subsoils. Better results are secured when the soil is moist than when it is either too dry or too wet. The best moisture conditions are about the same as for plowing. Good samples have been taken from silt loams, from sandy loams, and from clays. A sandy soil requires special care. Soils containing gravel, rock, or an abundance of coarse undecomposed organic matter cannot be sampled with satisfaction.

When taking samples of subsoil it is necessary to remove the surface soil from a place large enough to let the frame rest on the subsoil at the depth at which it is wished to start the sample.²

² Any one especially interested, can make arrangements with the Agricultural Experiment Station, Columbia, Mo., to secure blue prints and specifications for this sampler.

PLATE 1

Fig. 1. Sampler after removal from soil.

Fig. 2. Wrapped core of soil made air-tight with paraffine.



Fig. 1

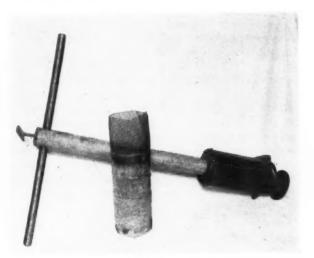
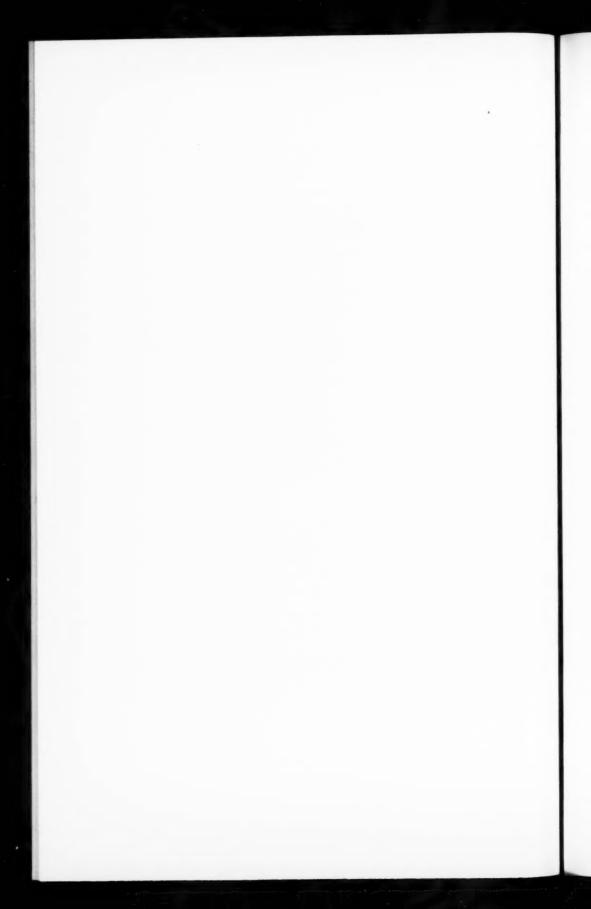


Fig. 2 57



THE AVAILABILITY OF NITROGEN IN GARBAGE TANKAGE AND IN UREA IN COMPARISON WITH STANDARD MATERIALS¹

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INTRODUCTION

The principal objects in the work reported in this paper are: First, to study the relative availability of garbage tankage in comparison with other organic and inorganic nitrogenous fertilizers; and second, to study the rate of decomposition of urea under various conditions, as well as to find out its relative availability.

The subject of garbage disposal has been and is still a matter of considerable importance, not only from the health point of view, but also from the economic standpoint. Garbage rendering plants have been in operation for the purpose of recovering from garbage, certain products such as fats, but no particular use has been found for the residue. Turrentine (10) points out that in the United States during 1914 the production of garbage tankage roughly estimated was 150,000 tons, valued at \$975,000. This was obtained from 1,200,000 tons of raw garbage rendered in about 25 plants. The garbage collected in 1909 in cities having a population of 30,000 or over, amounted to 2,700,000 tons, which would be capable of yielding 400,000 tons of dry tankage worth \$2,500,000.

Considerable work has been done in the past on the fertilizing value of garbage tankage, but most of the results seem to indicate that its nitrogen availability is low. Lipman and Burgess (4) found that garbage tankage gave results which place it in the same class with steamed bone meal, cottonseed meal, sludge from septic tanks, and goat manure.

Pember and Hartwell (6) showed that plants made no larger growth with garbage tankage as a source of nitrogen, than those to which no nitrogen was added. In chemical tests on the availability of garbage tankage they found that the alkaline permanganate method agreed more closely with vegetation tests than the neutral permanganate method. Further tests at the Rhode Island station (2) in the form of pot experiments showed that insoluble nitrogen in certain brands of fertilizers was practically useless and that the nitrogen of garbage tankage was of low grade.

Schroeder (9) also points out that from chemical tests made, it seemed that

¹ Paper No. 253 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

² The authors wish to express their indebtedness to Prof. A. W. Blair for suggesting the problem, and for helpful criticism of the work.

the proper use of garbage tankage should give the usual results obtainable from medium or low grade fertilizers. In another paper, Schroeder (8) reports results of pot culture tests compared with those obtained by the alkaline permanganate method. He concludes that a higher availability should be accorded the nitrogen in garbage tankage than would be indicated by the alkaline permanganate method.

1. VEGETATION TESTS

Pots of 2-gallon capacity containing 20 pounds of dry sand were used in this experiment. The general treatment of all the pots was as follows: 4 gm. of acid phosphate, 2 gm. of muriate of potash, 5 gm. of pulverized limestone, 0.5 gm. magnesium sulfate, and 0.2 gm. of ferric sulfate. The moisture content was kept at about 10 per cent. The source of nitrogen was the only varying or limiting factor. The different nitrogen treatments were as follows: (a) no nitrogen, (b) garbage tankage, (c) garbage tankage finely ground (d) sodium nitrate, (e) ammonium sulfate, (f) urea, (g) commercial synthetic urea,

TABLE 1

Yield of dry matter in the crops*

POT TREATMENT	BARLEY	RAPE	SORGHUN
	gm.	gm.	gm.
No nitrogen (check)	3.8	0.93	2.6
Garbage Tankage	6.7	2.00	10.0
Garbage Tankage finely ground	6.7	1.90	6.0
Nitrate of soda	36.5	17.70	26.8
Sulfate of ammonia	32.3	11.70	28.4
Urea (C.P.)	33.3	13.80	31.8
Commercial synthetic urea	34.6	13.50	30.6
Standard tankage	21.3	7.90	21.5
Fish	19.1	7.60	20.4

^{*} Average of 3 pots.

(h) standard tankage, (i) fish. All forms of nitrogen were applied at a rate equivalent to 2 gm. of nitrate of soda which actually contained 0.315 gm. of nitrogen. The garbage tankage analyzed 3.13 per cent total nitrogen. There were three pots for each nitrogenous material used. Two series of pots were prepared at the same time. Barley was planted in one series of pots and rape in the other. At a later date, another series of pots was prepared as above and sorghum was grown. The barley and rape were planted in the middle of February, and harvested the first part of May. The sorghum experiment was started May 4 and harvested July 6.

Yields of dry matter

The yields of dry matter for the different nitrogenous treatments with the three different crops are shown in table 1. With the barley crop, nitrate of

soda gave the largest yield, 36.5 gm., and garbage tankage the lowest, 6.7 gm. However, the yield in the garbage tankage treated pots was double that of the check, or no nitrogen pots. The two forms of urea yielded 33.3 and 34.6 gm. of dry matter, closely approaching the yield with nitrate of soda; and the sulfate of ammonia ran a close third with 32.3 gm. The same order holds true with the rape crop, and the standard tankage and fish gave yields about half as great as those from the sodium nitrate, with the preference for the standard tankage. With the sorghum plants, both forms of urea yielded 31 gm.; ammonium sulfate, 28.4 gm.; and nitrate of soda, 26.8 gm. It is also interesting to note that the garbage tankage, in this case, yielded three to four times as much as the no nitrogen pots. Pinckney (7) has pointed out that sorghum plants are good indicators of available nitrogen, and the appearance of these plants during their growing period seemed to bear out this fact. Attention may be called to the fact that the finely ground garbage tankage

TABLE 2

Percentage of nitrogen in the crops*

POT TREATMENT	BARLEY	RAPE	SORGHUM
	per cent	per cent	per cent
Nitrogen (check)	0.774	1.140	0.656
Garbage tankage	0.901	1.388	0.599
Garbage tankage finely ground	0.825	1.529	0.698
Nitrate of soda	0.732	1.080	0.670
Sulfate of ammonia	0.788	1.422	0.543
Urea (C.P.)	0.839	1.346	0.535
Commercial synthetic urea	0.760	1.331	0.591
Standard tankage	0.718	1.248	0.529
Fish	0.802	1.140	0.492

^{*} Average of 3 pots.

produced a considerably smaller crop of sorghum than the more coarsely ground garbage tankage.

Percentage of nitrogen in the crops

The percentages of nitrogen in the dry matter of the various crops are shown in table 2. The differences are not especially significant. The percentage of nitrogen in garbage tankage tends to run the highest in all of the crops, but this frequently happens when the crop yield is low, because of unavailable nitrogen. On the other hand, where there is a large yield of dry matter, as is the case with sodium nitrate, the percentage of nitrogen in the crop may be somewhat lower. This, however, does not hold true in the case of urea. Here the percentage of nitrogen is high with a high yield of dry matter, which would indicate that the nitrogen in urea is very readily available to plants.

Percentage of nitrogen recovered in the crops

The percentage of nitrogen recovered in the crops is shown in table 3. With the barley crop, it is interesting to note that the urea (C.P.) gave the highest percentage of nitrogen recovered, 77.37 per cent as against 75.49 per cent for nitrate of soda, even though the yield of dry matter was greater with the latter. The percentages of nitrogen recovered with commercial urea and nitrate of soda are about the same, the amount in each case being slightly more than the

TABLE 3

Percentage of nitrogen recovered in the crops*

POT TREATMENT	BARLEY	PAPE	SORGHUM
· · · · · · · · · · · · · · · · · · ·	per cent	per cent	per cent
No nitrogen (check)			
Garbage tankage	9.84	5.46	13.60
Garbage tankage finely ground	8.22	5.87	7.87
Nitrate of soda	75.49	57.33	51.60
Sulfate of ammonia	71.14	49.46	43.52
Urea (C.P.)	79.37	55.59	48.57
Commercial synthetic urea	74.16	53.68	52.00
Standard tankage	39.26	27.93	30.66
Fish	39.31	24.13	27.60

^{*} Average of 3 pots.

TABLE 4
Relative availability

Using 100 for nitrate of soda as a basis

POT TREATMENT	BARLEY	RAPE	SORGHUM	AVERAGE
	per cent	per cent	per cent	per cent
No nitrogen				
Garbage tankage	13.0	9.5	26.4	16.3
Garbage tankage (finely ground)	10.9	10.2	15.3	12.1
Nitrate of soda	100.0	100.0	100.0	100.0
Sulfate of ammonia	94.2	86.2	84.3	88.2
Urea (C.P.)	105.1	96.9	94.1	98.7
Commercial synthetic urea	98.2	93.6	100.7	97.5
Standard tankage	52.0	48.7	59.4	53.4
Fish	52.1	42.1	53.5	49.2

recovery with ammonium sulfate. Standard tankage and fish gave almost identical results as to amount of nitrogen recovered—39.26 per cent and 39.31 per cent respectively. The garbage tankage averaged about 9 per cent nitrogen recovered.

With the rape crop, nitrate of soda again resumed first place in amount of nitrogen recovered, with urea (C.P.) a close second, the amounts being 57.35 and 55.6 per cent respectively.

Commercial urea and nitrate of soda used with the sorghum gave almost

identical results. The other nitrogenous materials follow in the same order as under barley.

Table 4 expresses the relative availability of the various nitrogenous materials on the basis of 100 for nitrate of soda. In the last column, which shows the average of the three crops, urea (C.P.) is close to the nitrate of soda with 98.7 per cent available. Commercial synthetic urea rates 97.5 per cent, and sulfate of ammonia falls to 88.2 per cent. On this basis, the garbage tankage averages about 14 per cent; standard tankage, 53.4 per cent; and fish, 49.2 per cent.

Conclusions

It is evident from the foregoing data that the fertilizing value of garbage tankage is very low. Most of the nitrogen which it contains is slowly available, and the total amount of nitrogen which it contains is very low in comparison with other forms of organic nitrogen. As a fertilizing material, its chief value seems to be as a filler.

Urea was found to be a very desirable source of nitrogen and was very nearly equal to nitrate of soda in availability. In some cases the crop yield was even greater than with nitrate of soda. In all cases, it was better than with sulfate of ammonia.

2. CHEMICAL TESTS

Chemical tests for the availability of organic nitrogen have always been more or less failures. Of the two methods outlined in the Official Methods of Analysis (1), both usually give results which differ widely from the vegetation tests. The neutral permanganate method especially has been found by most workers to give values much too high. The alkaline permanganate method gives results that more nearly approach the vegetation tests, but the values obtained are also usually too high. A new method "Oxalic Acid" by Kellogg (3) has recently been tried out, and although far from perfect, gives results which are more in accord with actual vegetation experiments.

These three methods were tried out on the garbage tankage, on the standard tankage, and on the fish used in the above vegetation experiments. The results are given in tables 5, 6, and 7. The only values obtained which approximate those obtained in the vegetation tests are those secured by the Kellogg Oxalic Acid method. Even by this method the value for the standard tankage is much too low. By all three methods fish shows a higher availability than the standard tankage, but vegetation tests show the opposite to be true.

From the results of this work it would appear that the present chemical methods for determining available organic nitrogen are not satisfactory.

Decomposition of urea

The results of the vegetation tests reported above, indicate that urea is a very readily available source of nitrogen. The decomposition of the urea was

apparently very rapid even in sand cultures, for the plants showed no lack of nitrogen from the start. The authors decided to determine at what rates the urea was decomposed with different percentages of soil and sand. The recent work of Littauer (5) should be mentioned in this connection. He showed that the rapidity of urea decomposition was dependent on the kind of soil, soil

TABLE 5
Neutral permanganate method

MATERIAL	WATER- SOLUBLE	WATER-INSOLUBLE ORGANIC NITROGEN SOLUBLE IN NEUTRAL PERMANGANATE		WATER- INSOLUBLE ORGANIC NITROGEN INSOLUBLE IN NEUTRAL PERMANGA- NATE	TOTAL NITROGEN	PROPORTION OF TOTAL NITROGEN AVAILABLE
	per cent	per cent	per cent of total	per cent	per cent	per cent
Garbage tankage	0.40	1.88	60.0	0.85	3.13	72.8
Ordinary tankage	1.18	6.80	76.7	0.89	8.87	89.9
Fish	2.65	5.28	64.3	0.29	8.22	96.5

TABLE 6
Alkaline permanganate method

MATERIALS	WATER SOLUBLE			INACTIVE WATER INSOLUBLE ORGANIC NITROGEN		PROPORTION OF TOTAL NITROGEN AVAILABLE
	per cent	per cent	per cent of total	per cent	per cent	per cent
Garbage tankage	0.40	0.92	29.4	1.81	3.13	42.2
Ordinary tankage	1.18	4.77	53.8	2.92	8.87	67.1
Fish	2.65	3.53	43.0	2.04	8.22	75.2

TABLE 7
Kellogg's oxalic acid method

MATERIALS	WATER- SOLUBLE	WATER- SOLUBLE PLUS THAT SOLUBLE IN 0.01 N OXALIC ACID	INSOLUBLE IN WATER AND 0.01 N OXALIC ACID	TOTAL NITROGEN	PROPORTION OF TOTAL NITROGEN AVAILABLE
	per cent	per cent	per cent	per cent	per cent
Garbage tankage	0.40	0.65	2.48	3.13	20.8
Ordinary tankage	1.18	1.95	6.92	8.87	22.0
Fish	2.65	3.08	5.14	8.22	37.5

moisture, and temperature. In a loam soil the decomposition of urea was completed in 10 days at 20°, whereas it took 28 days at 0°. In sand cultures 65 per cent of the urea was decomposed in 10 days at 20°, and 20 per cent in 28 days at 0°. Littauer also states that drying limits urea decomposition in the same way as sandy soil. Furthermore, the decomposition of urea goes on more

rapidly in a soil rich in bacteria where there is sufficient moisture. In order to hasten the decomposition of urea and to prevent loss of ammonia, Littauer found it was necessary to mix the urea well with the soil, and not to expose it on the surface. Another interesting point brought out was that unlike nitrates, there is no danger of leaching, because ammonium salts are absorbed and held near the surface of the soil.

Experimental

In the experiments tried out on the rate of decomposition of urea with different percentages of soil and sand, tumblers containing 100 gm. of the dry soil or sand mixtures were used. About 10 per cent moisture was used in the sand tumblers, and about 15 per cent in the soil and half sand. An aliquot, equivalent to 0.1 gm. urea solution, was added to each tumbler and thoroughly mixed. This amount of urea contains 0.0462 gm. nitrogen. The cultures were maintained at room temperature. After standing different periods of time, the contents of each tumbler were transferred to a flat bottom 1-liter flask, and 500 cc. of a 10 per cent potassium chloride solution was added. This was shaken in a mechanical shaker for $\frac{1}{2}$ hour, and then filtered. Nitrogen was determined in the usual way by distilling 250 cc. of the filtered solution into standard acid. Since on distillation a small amount of ammonia is given off from a solution of urea, it was necessary to run a blank in all cases. Under some conditions, this amounted to 2 mgm. of nitrogen and under others to 3 mgm.

Discussion

The percentages of nitrogen converted to ammonia in sand, in half sand, and in soil cultures for each day are given in table 8. It is understood in all this work that part of the nitrogen from the urea had been converted to nitrates before the determinations for ammonia could be made, but this represents only a small part of the whole. The object of this work is to measure the rate of decomposition of urea by ammonification tests. Thus, in one experiment (table 8) after 5 days, only 3 per cent was converted to ammonia in the sand cultures, 67.1 per cent in the half sand culture, and 89.2 per cent in the straight soil culture.

When the experiment was repeated and carried through to 8 days, 6.9 per cent of the urea was converted to ammonia in the sand, 87.0 per cent in the half sand, and 92.2 per cent in the straight soil. With the soil alone, over 50 per cent was converted to ammonia within 3 days. In all of these experiments, 0.1 gm. of urea was used per 100 gm. of soil. With this or smaller amounts of urea, the soil was capable of absorbing and holding the ammonia produced but with larger amounts the soil could not absorb all the ammonia produced; therefore much was lost.

A further experiment was conducted to determine the rate of decomposition of urea in a very acid soil, and also in an acid soil to which varying amounts of pulverized limestone had been added. The soil chosen for this purpose was

one which had received a rather heavy application of ammonium sulfate for about 15 years but which had not been limed, and which had a pH value of about 4.8 at the time of the experiment.

Table 9 shows the results of the decomposition of the urea through a period of 11 days and under the conditions of no lime and of lime. Three different

TABLE 8

Rate of decomposition of urea

In per cent nitrogen converted to ammonia

PERIOD OF TIME	SAND	HALF SAND HALF SOIL	SOIL
1 day { 1		9.5	21.2
1 day \ 2	1.3	10.8	20.8
2 days { 1	3.0	24.3	46.0
2 days \ 2	1.3	23.8	39.0
, , 1	2.6	43.7	67.1
3 days { 1	1.8	36.0	60.0
, , ∫ 1	2.2	52.4	84.0
4 days { 1	2.6	47.2	79.0
5 days	3.0	67.1	89.2
7 days	5.2	87.9	92.2
8 days	6.9	87.0	92.2

TABLE 9

Rate of decomposition of urea in an acid soil

In per cent nitrogen converted to ammonia

PERIOD OF TIME	NO LIME	0.25 GM. CaCO ₈	0.5 gm. CaCO ₈	1 GM. CaCO
16 hours	5.8	6.2	6.7	10.4
2.5 days	15.8	37.7	43.1	54.5
3.5 days	19.0	41.9	60.0	70.5
4.5 days	26.2	60.4		77.7
5.5 days	30.8	71.8	81.3	83.8
7.0 days	34.5	66.5	84.6	87.5
7.5 days	40.5	73.6	85.7	88.3
1.0 days	52.6	88.3	91.4	85.3

quantities of limestone were applied to the tumblers; namely, $\frac{1}{4}$ -, $\frac{1}{2}$ -, and 1-gm. portions.

It will readily be seen that where no limestone was added, the rate of decomposition of the urea was slowed up greatly, and even at the end of 11 days, only about 50 per cent of the nitrogen was converted to ammonia. The application of the $\frac{1}{2}$ -gm. portion of limestone per 100 gm. of soil seemed to bring about the most rapid decomposition of the urea; 81.3 per cent of the nitrogen was converted to ammonia in $5\frac{1}{2}$ days and 91.4 per cent in 11 days.

SUMMARY

The object of this paper has been to study the relative availability of garbage tankage and urea in comparison with other organic and inorganic nitrogenous fertilizers, and to study the rate of decomposition of urea under various conditions.

Vegetation experiments were carried on in pots with sand cultures. Three crops were grown: barley, rape, and sorghum.

Comparisons of the various fertilizers are discussed under the headings: Yield of dry matter, percentage of nitrogen in the crops, percentage of nitrogen recovered in the crops, and the relative availability of each.

It was found that the fertilizing value of garbage tankage was very low. Most of the nitrogen which it contains is very slowly available, and its total percentage of nitrogen is also low in comparison with other forms of organic nitrogen. As a fertilizing material, its chief value will probably be in its use as a filler.

Urea was found to be a very desirable source of nitrogen and was very nearly equal to nitrate of soda in availability. In some cases the crop yield was even greater than with nitrate of soda. In all cases, it was better than sulfate of ammonia.

On the basis of 100 for nitrate of soda, urea rates 98 per cent available; sulfate of ammonia, 88.2 per cent; standard tankage, 53.4 per cent; fish, 49.2 per cent; and garbage tankage, 14.2 per cent.

Chemical availability tests were made by three different methods on three of the organic fertilizers, and were compared with the vegetation tests. The conclusion was reached that, except in a general way, not much dependence can be placed on the present chemical methods for the determination of available organic nitrogen.

The rate of the decomposition of urea was studied in cultures of sand, of a mixture of half sand and half soil, and of soil alone. The index for determining this was the amount of nitrogen converted to ammonia from the urea, over different periods of time. The cultures were maintained at room temperature. After 5 days, only 3 per cent was converted to ammonia in the sand culture, 67 per cent in the half sand cultures, and 90 per cent in the soil alone. With the soil alone, over 50 per cent was converted to ammonia within 3 days.

On an acid soil, the rate of the decomposition of the urea was very much retarded, and even at the end of 11 days, only about 50 per cent of the nitrogen was converted to ammonia.

The application of various amounts of limestone to the acid soil increased proportionately the rapidity of urea decomposition.

N

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PLATE 1

- Fig. 1. Comparison of Different Nitrogenous Fertilizers—Barley
 - 1. No nitrogen
 - 2. Garbage tankage
 - 3. Animal tankage
 - 4. Urea
 - 5. Sulfate of ammonia
 - 6. Nitrate of soda

FIG. 2. COMPARISON OF DIFFERENT NITROGENOUS FERTILIZERS—SORGHUM

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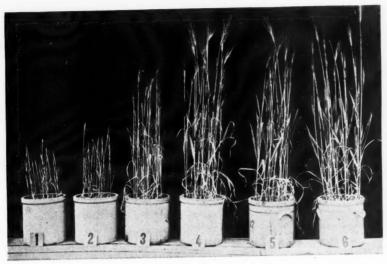
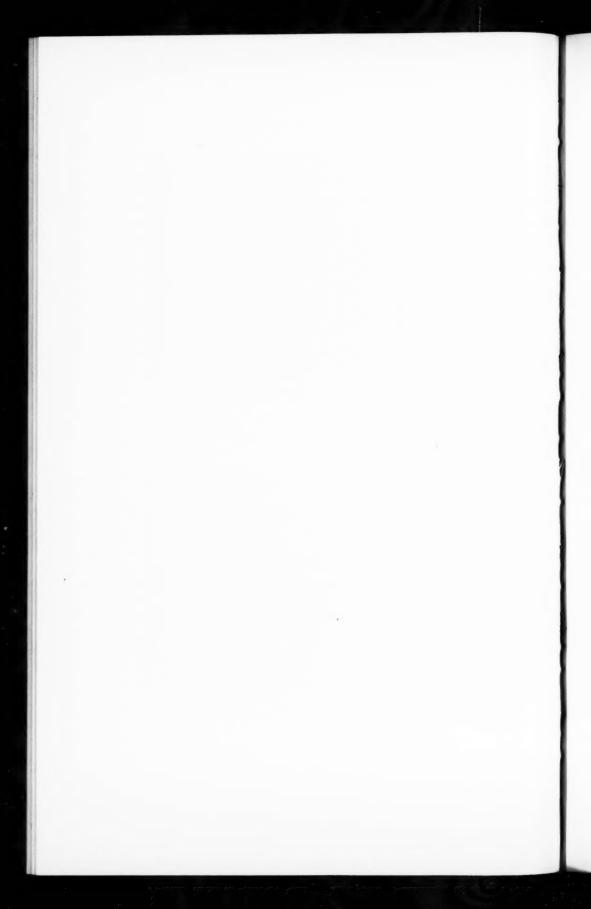


Fig. 1



Fig. 2



SOME RESIDUAL EFFECTS OF NEUTRAL SALT TREATMENTS ON THE SOIL REACTION

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More complete data concerning the chemical constitution of soils are not only a prerequisite to a better knowledge of the nature and extent of the soil reactions that take place under different soil treatments but also an aid to an understanding of the economic relationships of these reactions.

This article deals with the effects, on the soil reaction of the various horizons of four soil type profiles, of some different cations fixed by these soils from neutral salts (chlorides), after the soluble products of the soil-neutral salt reaction have been practically completely washed from the soils. It is intended to call attention principally to the results obtained on the particular soils under investigation, and also to show the remarkable agreement in the nature of these results among the different soils, which is not usually encountered in chemical soil investigations. In addition to the foregoing points, more evidence as to the possible combination of the fixed cation in the molecule of the soil compound responsible for the effects observed is set forth in this report.

SOILS USED

For this study, four types of soils were selected; namely, Miami loam, Kewanee silt loam, Nappanee silt loam, and Bellefontaine sandy loam. Typical profiles for each soil were selected.¹ The surface, or A horizon, sample was taken to a depth of 6 inches from a cultivated field adjacent to the place where the remainder of that particular profile was obtained. All the other samples are from horizons of virgin, forested soils, and were taken, not on a basis of inapplicable linear depths, but as directly representing the various well-marked soil horizons present in the several types examined. The A₂ and A₃ horizons are those of elutriation, which is brought about by the soil weathering processes, and are characterized chiefly by a relatively low content of easily soluble bases. A part, at least, of the elutriated materials from the A horizons has been concentrated in the soil portion directly beneath them and has formed the distinct soil layers designated as the B horizons. The C horizons designate the parent, or unweathered, soil material presumably as deposited by glacial

 $^{^{1}\!}$ Complete descriptions of these type profiles may be obtained from recent Michigan Soil Survey Reports.

action; the soil samples representing these horizons were taken at some distance below the visible zone of soil weathering.

PROCEDURE

The soil samples were dried in the air, passed through a 2-mm. mesh sieve, and thoroughly mixed to make them uniform in composition. For the soil treatments, 2N solutions of CaCl₂, MgCl₂, KCl, and NaCl were used, the CaCl₂ and MgCl₂ solutions being made neutral before adding them to the soils.

Six 20-gm. soil charges were weighed out for each horizon, placed into small beakers, and given the following respective treatments; check, washed check, CaCl₂, MgCl₂, KCl, and NaCl. The check sample was placed into an 8-ounce sterilizer bottle with 50 cc. of water and shaken constantly for 7 hours. The salt treated samples, which received 50 cc. each of the 2N salt solutions, were allowed to stand with frequent stirring, for 1 hour, then they were thrown on a filter paper and washed with water until chlorides were absent from the leachings, transferred to 8-ounce sterilizer bottles with 50 cc. of water each, shaken for 5 hours, and left to stand over night. The washed check sample of soil received the same amount of washing and the same subsequent treatment as did the salt treated samples. The following morning the hydrogenion concentrations of the several samples were determined by means of the hydrogen electrode.

DISCUSSION

The data are shown graphically in figure 1. The vertical lines, from left to right, represent pH values, the pH value 7 being indicated by a heavy line. Each set of six treatments represents one soil horizon. The results of the several horizons are placed in the same relative position on the graph as that occupied in the soil profile. The variation in the pH values of the various horizons and the corresponding residual effects of the neutral salt treatments are easily observable.

The washed check treatments were introduced in order to determine if the effects of washing the soil samples would be great enough to interfere with any conclusions that might be drawn from the results of the salt treatments; and it is believed that the observed effects of these treatments on the several soil samples are not due to the washing of the soils.

Perhaps the most important point in connection with this research is the consistent relationship found in the results of the four salt treatments—CaCl₂, MgCl₂, KCl and NaCl—as compared with the check treatments. The residual effects of the CaCl₂ treatments were small; this salt caused no great change in pH values over those of the check samples, and was less effective in changing the soil reaction than any of the other salts. Next in order of magnitude are the MgCl₂ effects, a consistent increase in pH values being found over the check and CaCl₂ treatments. The KCl and NaCl treatments

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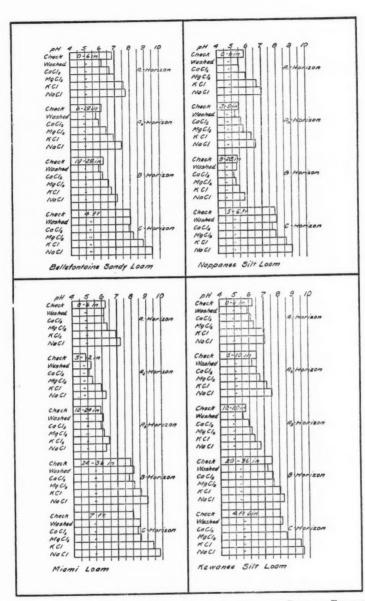


Fig. 1. Variation in pH Values of Horizons and Corresponding Residual Effects of Neutral Salt Treatments on Four Soil Types

caused marked increases in pH values in all of the horizon samples, in some cases even making acid soils alkaline in reaction; the NaCl effects were consistently greater than those of KCl. Hence, the order of the several cations with regard to their residual effects on increasing the pH values of the soils is: Ca, Mg, K, Na.

Two other points of importance are that the salt treatments produced relatively the same effects on the alkaline soil samples as on the acid samples; and that the acidity produced in soils by neutral salt treatments is soluble and can be leached readily from the soils.

Some soil investigators hold that the neutral salt cation fixed by soils is selectively adsorbed on the surface of the soil particles (colloids), thus setting free the anion of the salt, which in turn increases the hydrogen-ion concentration of the solution; whereas others claim that this reaction between soils and neutral salts is a chemical reaction involving the equivalent exchange of cations between the soils and salt. No attempt will be made here to discuss the merits of the foregoing theories, but our data tends to support the chemical theory because: (a) A cation relationship is shown in that the CaCl₂ salt caused very little change in the soil reaction; and (b) Since the soluble acids and other soluble products were washed from the soils, the increase in pH value is best explained on the basis of increased OH-ion dissociation due to greater solubility and hydrolysis of the soil material containing the fixed cations. In these soils, the principal reacting base is calcium; hence, when they are treated with CaCl₂ little exchange of cations can take place, and the soils are not markedly changed with respect to their bases or basic properties. However, when other cations, particularly potassium and sodium, are substituted for the soil calcium, marked changes in the basic properties of the soils are observed

SUMMARY

- 1. Soil samples from the horizons of four soil profiles were treated separately with the neutral salts CaCl₂, MgCl₂, KCl and NaCl, in order to determine the residual effect on the soil reaction after the soluble products of the soil-salt reaction were washed from the soils.
- 2. The CaCl₂ treatment caused only slight changes in the soil reaction, whereas the MgCl₂, KCl, and NaCl treatments increased the pH values of the soils. The effective order of the several cations is: Ca, Mg, K, Na.
- 3. Increased solubility and hydrolysis of the soil material containing the fixed cation are believed to be the cause of the increased pH values where increases are noted.

THE UTILIZATION OF WATER BY PLANTS UNDER FIELD AND GREENHOUSE CONDITIONS

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An understanding of the characteristic features of the utilization of soil moisture by plants during their growth period is of great importance to the farmer in the arid region, in order that he may regulate this process. The Saratov Experiment Station, therefore, during the year 1924, made parallel observations on the utilization of water by plants under field and greenhouse conditions. For this purpose, in the field experiments most of the field crops were used, whereas in the greenhouse, on account of lack of space, fewer crops—of the most representative plants—were utilized.

METHODS OF OBSERVATION

In order to investigate the utilization of the soil moisture by plants, crops on typical soils were taken, under normal conditions of culture. The following plants were used in the field: winter rye and wheat, soft and hard spring wheat, oats, and barley. From the leguminous plants the following were used: lentils, peas, and noot; from the grasses: sorghum, Sudan grass, and alfalfa; from the tillable and oily crops: buckwheat, corn, sunflower, potatoes, carrots, pumpkin, and flax.

In the greenhouse the following crops were used: soft and hard spring wheat, oats, buckwheat, peas, clover, flax, sunflower. For each of these plants the number of duplicate pots used was such that sampling the soils at 10-day intervals during the vegetation period sufficed. A moisture content equivalent to 60 per cent of the moisture-holding capacity was maintained during the growth period.

The plants in the greenhouse were sown at the same time as the plants in the field; the samplings were done at 10-day intervals. The portion of the plant above the ground was weighed in the wet state soon after cutting, dried, weighed again, and analyzed for ash, nitrogen, and phosphoric acid.

The daily losses of moisture as determined by comparing the weight of the pots and the plants during the growth period with the weight of the dry matter of the crop, afford a means of determining accurately the utilization of water by the plants during the different periods of growth.

¹ Translated from the Russian manuscript by J. S. Joffe, New Jersey Agricultural Experiment Station.

In the field, moisture determinations were made on corresponding days at a depth of 100 cm. over an area of 10 cm.; the crops from definite areas upon harvesting were weighed, dried, and weighed again. The crop samples were analyzed for ash and nitrogen.

METEOROLOGICAL PECULIARITIES

The vegetation period during the year was typical for the dry area around the lower Volga. The summer was exceptionally hot and dry. The rainfall during the whole period of growth of the early spring grains was very slight, but heavy showers fell on June 5. In the field the plants were growing primarily on the moisture reserve from fall and winter; for that reason the growth was far from being satisfactory and the yield therefore was diminished. The plants in the greenhouse did not suffer from a lack of moisture, but the comparatively low moisture content of the air and the high temperature had its effect on their development.

The temperature of the air in the greenhouse for the 10-day period from June 10 to June 19 was, on the average, 28.1°C.; the relative humidity of the air was, on the average, 36 per cent. This was the hottest and driest 10-day period during the growth of the wheat. The second half of the vegetation period was more favorable for growth; therefore the late tillable crops developed and gave a normal yield.

POLTAVKA WHEAT IN FIELD AND IN GREENHOUSE

The wheat was planted on April 23 and came up on May 4. Stooling was noticeable on May 19; heading out, about June 15 or 16; the crop matured about July 9 or 10. It is interesting to note that the development of the wheat in the greenhouse and in the field was absolutely identical, notwithstanding the sharp differences in the condition of their growth—heavy watering in the greenhouse, on one hand, and the typical drought of the soil in the field. Simultaneous samplings of the growing plants were made on May 20 and 30, June 9, 19 and 30, and on July 10 and 20.

To calculate the transpiration coefficient in the field, use was made of the figures on the amount of water lost from the soil as determined by difference between two successive determinations of the moisture content at the depth of 100 cm. excluding the amount of rainfall. It was supposed that the latter was fully utilized by the soil, increasing in one way or another the percentage of moisture in it. Notwithstanding the fact that a part of the water was indirectly evaporated from the soil surface, and therefore this supposition was not exactly right, there was no possibility of approaching the problem in any other way. The transpiration coefficient in the greenhouse was determined in the ordinary way, that is, by dividing the total amount of water evaporated during the growth period by the weight of the dry yields. The fundamental data on this question are given in table 1.

The data presented for the field and greenhouse experiments suggest that the processes of the growth of Poltavka wheat run very closely even under such variable conditions. Of course, with the greenhouse experiment one gets a more distinct picture of the growth and also of the various limitations, since the plants themselves and the moisture supplied are fully controlled by the experimenter. It is entirely different in the field. Even the increase of growth cannot be discounted, since no two neighboring plots ever have the same vegetation. Undoubtedly there is a similar variation in the pots, but this difference is not so great.

It is especially difficult to calculate the amount of soil water utilized by plants. In the first place it is not simple to calculate the moisture which is being evaporated from soils. There may be individual departures in different

TABLE 1

The amount of tops, utilization of water, and transpiration coefficient of Poltavka wheat in various phases of its growth

TIME PERIOD OF OBSERVATION	YIELD OF TOPS		DRY MATTER		AMOUN	SPIRED	WATER EVAPO- RATED DUR- ING THE			TRANSPIRA-		
					At each 10-day interval		Total		10-	DAY	COEFFI	CIENT
	m. In field	In greenhouse	per Lent	per cent	poods to be poods	In greenhouse	poods poods	In greenhouse	per cent	In greenhouse	In field	In greenhouse
May 20	16	1.8		22	39,435	508	39,435	508	28		3,933	282
May 30		5.5	-	15	,		63,181			12	2,256	362
June 9	120	13.2	27	21			79,327		12	25	1,259	384
June 19	117	21.1	44	35	34,732	3,442	114,059	8,522	25	28	1,141	402
June 30	164	23.8	52	45	21,733	2,187	135,792	10,709	16	18	823	449
July 10	79	21.5	66	69	3,693	1,445	138,485	12,154	3	12	1,395	565

soils with variable degrees of porosity. It is absolutely impossible to determine what portion of the rainfall moisture evaporates indirectly from the surface of the soil and does not take part in the processes of transpiration of plants.

To simplify matters it is necessary to take into consideration all the atmospheric precipitation, assuming that it takes part in one way or another in the formation of that portion of moisture which is found in the soil at the time of sampling.

It is interesting, however, to note that even with a crude method of calculation, very close similarity was found in the processes of transpiration in the field and in the greenhouses. Discarding the first two 10-day periods, when the plants were still small and did not cover the soil, and when the moisture,

therefore, could evaporate freely from the surface, it was found that later there was a true parallel in the evaporation from the plants in the field and in the greenhouse. With a gradual increase the transpiration of wheat reached its maximum, both in greenhouse and in field, during the 10-day period from June 9 to 19, when heading out and blooming took place.

As may be inferred from the data given, Poltavka wheat, with constant watering of the soil in the greenhouse, transpired for the 20-day period—which coincides with the formation of stems, heading, and flowering—a little more than half of all the moisture necessary (53 per cent) for its growth. For the whole month of June the wheat transpired 71 per cent of the moisture transpired for the whole vegetation period.

From this we may see clearly the significance of rainfall during May and the beginning of June: it is from this source that the wheat draws its moisture for development.

Under field conditions matters are somewhat different. Here under the conditions of drought during 1924 a great quantity of moisture from the soil was lost during the first days of the development of the wheat. The moisture so lost was unavailable for the plants; the losses came about not through the plants, but indirectly, through the soil.

If, during the first two 10-day periods of the development of the wheat in the greenhouse, only 16 per cent of the total amount of moisture necessary for this development evaporated, then the unproductive loss of water from the soil for the same period is equivalent to 30 per cent of the total amount. Under such conditions the necessity of having a large amount of soil moisture in the spring for the successful growth of wheat is sharply brought out. As the soil is being covered with plants and shaded, evaporation of moisture through the soil is prevented; at this time the amounts of water necessary for the growth of the plants both in the greenhouse and in the field are very nearly equal. The great difference in the third 10-day period when less water was apparently evaporated in the field than in the greenhouse, is explained by the fact that during this time there was a heavy downpour of rain which gave to the soil 32 mm. of water, increasing the soil water content at the time of the determination.

Much less similarity is found in the rate of growth under field and greenhouse conditions. The difficulties in taking uniform samples of plants in the field become apparent, and the growth increase in the field does not correspond to the increase in the greenhouse. Notwithstanding the greater soil moisture consumption during the period of June 9 to 19, during which an increase in growth should appear, there was none in the field, although in the greenhouse during the same period the increase was quite appreciable. A large discrepancy may be accounted for by the fact that the samples were taken when ripe. Although in the greenhouse the last sampling was somewhat larger than the one previous, in the field the last sampling was only half of the one previous; this introduces a great error.

If the decrease in dry matter of the field crop had been proportional to that

in the greenhouse, then the yield of the last sampling would have been about 150 gm.

The discrepancy in taking the field samples greatly influences the calculations of the transpiration coefficients; they cannot therefore be the same both in the field and in the greenhouse. The last two columns of table 1 give an idea of the amount of moisture necessary for plant growth in the different vegetative periods. It also gives the transpiration coefficient for the total yield.

The figures on the transpiration coefficient is the greenhouse, show that transpiration increases with every 10-day period, the matured plant doubling that for the first sampling. The data lead to the supposition that the formation of the vegetative mass of Poltavka wheat in the different periods of its growth does not coincide with the loss of moisture. The earlier portions of the plant seem to require for their formation less water than the portions which form later. As the plant is developing, each new unit of dry matter is formed with greater difficulty than the previous one.

After all, the transpiration coefficient of Poltavka wheat in the greenhouse during the year of the experiment was what it should be in a dry year.

The data on the field observations give an entirely different picture. If the weight discrepancy of the last sampling is connected in proportion to the crop from the greenhouse, the transpiration coefficient for the total yield decreases appreciably and equals about 920. The change of the unit of the transpiration coefficient in the field at maturity produces a picture in perfect contrast to that in the greenhouse. Beginning with the large figure 3900, it gradually falls at maturity and is decreased approximately four times as compared with the first value.

If the correction is not made, the transpiration coefficient in the field will be approximately two and one-half times as great in the field as in the greenhouse. With the correction for the crop, this value is decreased gradually until it is only twice as great. At any rate the utilization of water by wheat in the field during the year 1924 was twice that in the greenhouse which must be ascribed to the indirect loss of soil water, which was not taken into consideration.

The figures on the transpiration coefficient of the first 10-day period bring out this point. In the first 10-day period the wheat in the field is losing nearly 14 times as much water for each unit of dry matter as that in the greenhouse. In the next 10 days this loss is cut 2; the next, 2 more; gradually approaching to only 2 or 3 times the amount, as compared with the greenhouse. We must assume that if it would be possible to eliminate the indirect loss of water from the field soil, the utilization of water for the formation of dry matter of the crop in the field and in the greenhouse would not differ very much.

BELOTOORKA WHEAT IN THE FIELD AND IN THE GREENHOUSE

With simultaneous plantings the germination and the first phases of development of Belotoorka and Poltavka were the same. A noticeable backwardness

begins with Belotoorka during the phase of heading; Belotoorka matures 10 days later than Poltavka, and the last sampling was taken July 20. The time periods of taking the samples were the same. The data for Belotoorka are presented in table 2.

The conditions of growth of Belotoorka in the field and greenhouse, are comparable to those of Poltavka. Notwithstanding the inconsistency in the figures for the field plants, we still obtain a comparable picture for the field and greenhouse, and for the evaporation of the water from the soil.

The evaporation of water from the field soil with Belotoorka is small during the second 10-day period but is very large during the third. It is possible that there was an error in determining the moisture content of the soil.

The water requirement for Belotoorka wheat in the field and in the greenhouse was at its maximum during the first 20 days of June. In the field,

TABLE 2

The amount of tops, utilization of water, and transpiration coefficient of Belotoorka wheat in various phases of its growth

TIME PERIOD	YIELD OF TOPS		DRY MATTER		AMOU	SPIRED	WATER EVAPO- RATED DUR- ING THE		TRANSPIRA- TION			
					At each 10-day interval		То	tal	10-	DAY	COEFF	CIENT
OF OBSERVATION	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse
	gm.	gm.	per cent	per cent	poods	poods	poods	poods	per cent	per cent		
May 20	4	1.6	23	19	26,835	526	26,835	526	19	7	3,354	337
May 30	15	4.8	22	15	5,266	1,284	32,101	1,910	4	17	1,107	378
June 9	28	13.6	29	22	57,306	2,739	89,407	4,549	41	37	1,687	336
June 19	75	14.9	36	42	37,252	1,802	126,659	6,351	26	24	880	426
June 30	88	16.0	45	51	3,253	966	129,912	7,317	2	13	770	457
July 9	85	18.4	61	67	6,213	105	136,125	7,422	4	1	835	403
July 19	101	16.3	63	78	4,680	40	140,805	7,462	3	5	725	458

67 per cent and in the greenhouse 61 per cent of the total for the whole period of growth were evaporated. It is worth noting that the process of utilization of water during the growth of Belotoorka is somewhat different from that of Poltavka. In the greenhouse the Poltavka up to June 1 took only 16 per cent of the total moisture necessary, while Belotoorka for the same period took 24 per cent. The maximum utilization of water by Poltavka was during the period from June 9 to 19. For Belotoorka this maximum was 10 days earlier. For that reason up to June 9, 61 per cent of the total moisture was necessary for Belotoorka whereas for Poltavka only 41 per cent was necessary. Still the development of Belotoorka was slower than that of Poltavka. Apparently

Belotoorka requires for successful growth a good deal more water in the beginning than Poltavka.

As to the accumulation of dry matter in the field, the Belotoorka was more successful than the Poltavka, consequently the transpiration coefficients for the 10-day periods were more regular. The greatest transpiration coefficient, which was in this case also during the first 10-day period, is equal to 10 times the coefficient for the greenhouse. This very clearly indicates the great loss of water from the soil under wheat during the first weeks of its development when it does not as yet shade the ground and the moisture evaporates without any hindrance from the soil surface.

After two 10-day periods the transpiration coefficient of Belotoorka drops to half; in the next 10-day period to another half; and after that it changes but little up to maturity. Because of the accuracy of sampling at harvesting, the transpiration coefficient for Belotoorka was a good deal less at this time than for Poltavka and relatively close to the calculated value of Poltavka. A comparison of the transpiration coefficient in the field with that in the greenhouse, shows that the latter was fluctuating within very small limits, gradually increasing at maturity. It is interesting to note that during the year of observation the transpiration coefficient of Belotoorka in the field and in the greenhouse was appreciably less than for Poltavka. The total amount of water evaporated during the growth period of these two kinds of wheat was the same in the field, but in the greenhouse the Poltavka evaporated more water than Belotoorka.

VICTORIA PEAS IN THE FIELD AND IN THE GREENHOUSE

Among the legumes, Victoria peas planted in the field and in the greenhouse at the same time on April 29 were observed in both places. The rate of growth of the peas in the field and in the greenhouse was the same; during June 23 and 25 both sets of plants bloomed. In the greenhouse the peas were harvested on July 22, and in the field on July 29. The data for the Victoria peas are shown in table 3.

The character of growth of the peas in the greenhouse and in the field is about the same; there is a gradual accumulation of dry matter almost to the end of the development and a slight decrease before harvesting. The pea crop in the field developed later than in the greenhouse. In the utilization of soil water by the plants in the field and in the greenhouse, there was very little similarity, but this must be ascribed to the method of determination, which is not at all satisfactory under field conditions. Here, just as in the previous cases, during the first interval the peas in the field lost a large amount of water on account of the indirect evaporation of water from the soil surface. Later a greater utilization of water by the field plants, especially during the first 10 days of June, was noticed. This corresponds with the blooming period of the peas. In the greenhouse the utilization of water was more regular and more or less equal during the whole period of growth. The very early and the very

TABLE 3

The amount of tops, utilization of water, and transpiration coefficient of Victoria peas in various phases of its growth

TIME PERIOD OF OBSERVATION	YIELD OF TOPS		DRY MATTER		AMOU	SPIRED	WATER EVAPO- RATED DUR- ING THE		TRANSPIRA- TION			
					At each 10-day period		Total		10-	DAY	COEFFI	CIENT
	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	in greenhouse	In field	In greenhouse	In field	In greenhouse
-	gm.	gm.	per cent	per cent	poods	poods	poods	poods	per cent	per cent		
May 22	4	3.5	16	16	13,507	532	13,507	532	14	2	1,688	151
June 2	15	7.8	18	13	6,106	3,111	19,613	3,642	6	13	676	466
June 11	38	13.3	17	16	40,506	3,866	60,119	7,509	41	16	847	562
June 21	52	21.1	24	23	9,532	5,966	69,651	13,475	10	25	697	639
July 1	62	33.8	52	24	9,133	4,124	78,784	17,599	9	17	662	520
July 11	87	44.9	32	30	4,533	4,187	83,317	21,786	5	18	500	484
July 21	108	36.2	45	50	4,860	1,917	88,177	23,703	5	8	426	655
July 29	98		71		10,330		98,507		11		524	

TABLE 4

The amount of tops, utilization of water, and transpiration coefficient of buckwheat in various phases of its growth

TIME PERIOD	YIELD OF TOPS		DRY MATTER		AMOUNT OF WATER TRANSPIRED BY PLANTS					WATER EVAPO- RATED DUR- ING THE		SPIRA- ON
					At each 10-day interval		Total		10-	DAY	COEFF	CIENT
OF OBSERVATION	In field	In greenhouse	In field	In greenhouse	In field	In greenbouse	poods	In greenhouse	per In field	In greenhouse	In field	In greenhouse
May 30	gm.	gm.	cent	cent 8	poods	62	pools	62	cent	Cent C.4		65
June 9	22	15.4	15	18	23,998		23,998		22	13	571	136
June 19		19.7		14	21,292					23	285	296
June 30		41.6	28	23	41,053	3,494	86,343	9,345	38	22	381	224
July 10	1	52.0	32	30	5,373	3,705	91,176	13,050	5	23	226	250
July 20	156	78.2	30	47	1,500	2,244	93,216	15,294	1	14	312	195
July 31	214	80.3	35	65	1,930	738	95,146	16,032	2	5	232	200

latest phases of growth of the greenhouse peas required a small amount of water; it seems that during the period before blooming, peas require the greatest amount of soil moisture. There is a great uniformity in the transpiration coefficients of the peas both for the field and for the greenhouse conditions,

except that the greatest transpiration coefficient is during the first period of growth in the field whereas it is the smallest during the same time in the greenhouse. Later these values are not so far apart and in the final results it may be shown that the transpiration coefficient in the field is even less than in the greenhouse.

BUCKWHEAT IN THE FIELD AND IN THE GREENHOUSE

In the observations with buckwheat during 1924, the last sampling at maturity was not taken and therefore a comparison of the final transpiration coefficients of this plant at maturity is impossible. Neither were the observations for the growth and water utilization in the field and in the greenhouse made at the same time, the observations in the field being two days later. These data are summarized in table 4.

Unfortunately there was no sample at harvesting and therefore no possibility of determining the transpiration coefficient at harvesting. It is, however, very interesting to note that the transpiration coefficients of buckwheat in the field and in the greenhouse are very close, beginning with the third sampling.

As has been repeatedly shown in experiments on the transpiration coefficient of tillable plants, buckwheat belongs to the plants with a comparatively small transpiration coefficient. This was confirmed by observations in the field and in the greenhouse during 1924. The buckwheat required the least amount of water for its growth, giving at the same time a very large amount of growth at harvesting.

The utilization of water in the greenhouse was regular during almost the whole growth period, and it was only during the last 10-day period before maturity that the plants required a relatively small amount of water. It was not so regular in the field. A fairly large amount of moisture—about 12 per cent—was used by the plants during the last 10-day period (from August 1 to 11), whereas in the greenhouse during the same period only 5 per cent was used.

Notwithstanding the crudeness of the determination of the utilization of water by plants during their growth in the field, there was a chance to note very accurately the characteristic properties of buckwheat in this respect. This supports the belief of the author that by observing plants in the field it is possible more or less, although in relative figures, to notice the character of the utilization of soil water by crops. On the other hand the greenhouse experiments may serve to differentiate in series the cultural plants from the standpoint of their utilization of water even under field conditions.

SUNFLOWERS IN THE FIELD AND IN THE GREENHOUSE

Parallel observations on sunflowers grown under field and under greenhouse conditions were made, with the greenhouse plants started somewhat earlier. The ripening of the sunflowers in the field was delayed and the harvest was completed 10 days after the greenhouse crop was harvested.

Table 5 gives the data on the sunflower plants. In determining the transpiration coefficient of sunflowers, one must consider the difficulties connected with the selection of samples for the determination of the dry matter. The difficulty consists in that one must take only five to eight plants. To select more or less uniform plants every time is difficult, and therefore a series of irregular figures is obtained for the dry matter. This is particularly noticeable at the end of the vegetation period when it is necessary to deal with the large plants. It is impossible to avoid this in the greenhouse; for that reason the dry matter figures of the crop vary from time to time.

In the greenhouse there is a regular increase of the utilization of water with the growth of the plant, whereas in the field for the period from June 11 to July

TABLE 5

The amount of tops, utilization of water, and transpiration coefficient of the sunflower plant in various phases of its growth

TIME PERIOD	YIELD OF TOPS		DRY MATTER		AMOUN	SPIRED	WATER EVAPO- RATED DUR- ING THE		TRANSPIRA- TION			
					At each 10-day interval		Total		10-	DAY	COEFF	ICIENT
OF OBSERVATION	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenbouse	In field	In greenhouse	In field	In greenhouse
	gm.	gm.	per cent	per cent	poods	poods	poods	poods	per cent	per cent		
June 2	17	5.6	15	11	12,892	2,167	12,892	2,507	8	7	402	449
June 11	63	14.4	14	13	59,826	4,146	72,718	6,653	36	14	605	462
June 21	72	26.2	18	18	32,212	6,031	104,930	12,684	19	20	760	484
July 2	112	41.5	22	19	30,973	4,404	135,903	17,088	19	15	635	412
July 12	160	56.1	24	22	4,063	5,978	139,996	23,064	2	20	455	411
July 22	130	53.8	20	22	3,687	3,522	143,653	26,586	2	14	576	494
August 1	146	67.4	27	33	1,053	2,085	144,706	28,671	1	7	516	425
August 11	200	50.8	33	33	5,040	1,640	149,746	30,311	3	5	390	595
August 22	190		37		15,567		165,313		9		454	

22, the variations in the amount of water utilized for 20 days are not so great. In the field the great mass of water from the soil was taken in the beginning of July. After that period very little water was utilized.

The observations recorded bring out the very interesting fact—the transpiration coefficients of sunflowers, in the field and in the greenhouse are very close to each other—that the development of sunflowers requires somewhat more water in the field than in the greenhouse. The final transpiration coefficients are 595 for the greenhouse and 554 for the field.

From the figures it may be inferred that the sunflower plant requires a large amount of moisture for its development in the field. Of course this may be explained by the prolonged vegetation period, which in this case was

a month and a half greater than the vegetation period of wheat. This affords the sunflower, and other plants with a prolonged vegetation period, the possibility of utilizing the rainfall of the second half of the summer when the spring wheat ceases to develop. For that reason the yield of these plants does not depend so much upon the spring rains and soil moisture; these are not dependable in that neighborhood.

Notwithstanding the fact that the soil under the sunflower plant, generally speaking, evaporates a great amount of moisture, the transpiration coefficient of the sunflower is not so great. This may be explained by the fact that sunflowers give a large yield of tops. The figures that are available for sunflowers indicate that during the vegetation period about 75,000 poods² per desiatina³ of rain water fell.

TABLE 6

The amount of tops, utilization of water, and transpiration coefficient of flax in various phases of its growth

TIME PERIOD OF OBSERVATION	YIELD OF TOPS		DRY MATTER		AMOUN	SPIRED	WATER EVAPO- RATED DUR- ING THE		TRANSPIRA- TION			
					At each 10-day interval		To	tal	10-	DAY	COEFF	ICIENT
	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse	In field	In greenhouse
	gm.	gm.	per cent	per cent	poods	poods	poods	poods	per cent	per cent		
May 22	5	0.6	15	14	10,987	310	10,987	310	11	1	1,099	508
June 2	16	3.7	23	17	33,826	2,248	44,813	2,558	35	9	1,446	697
June 11	28	7.7	22	18	19,506	4,143	64,319	6,701	20	17	1,191	874
June 21	30	12.7	36	22	17,932	6,221	82,251	12,922	18	25	1,418	1,018
July 1	33	20.3	68	25	13,333	3,756	95,584	16,678	14	14	1,518	820
July 11	27	26.2	90	30	1,173	4,871	96,757	21,549	1	19	1,861	823
July 22		28.7		33		3,397		24,946		14		869

Calculations show that the sunflower plants evaporated during their period of growth approximately 165,000 poods of water per desiatina. Thus the sunflower had at its disposal from the precipitation about 46 per cent of the moisture required; it was forced to obtain 54 per cent from the moisture stored in the soil.

FLAX IN THE FIELD AND IN THE GREENHOUSE

Among the other oily plants for the region, flax has a certain significance, although it is not planted extensively in the district.

² Pood = about 36 American pounds, or 16.38 kgm.

⁸ Desiatina = about 2.7 acres.

Samples of flax were taken beginning with May 21. The hot weather and the lack of rain stimulated so its growth in the field that around July 11 harvesting was necessary. The greater moisture content of the greenhouse soil retarded the development of the flax and its maturity was delayed 10 days.

Table 6 gives the data for flax.

The general trend of accumulation of dry matter of the field crop is parallel to that in the greenhouse, although, as was mentioned, the flax in the greenhouse was retarded in its growth as compared with the flax in the field. Due to the fact that the soil moisture content in the greenhouse was constant and fairly high, the greenhouse plants contained a greater amount of moisture than the field plants, which were practically dry at the time of harvest.

The accumulation of dry matter in the field plants was completed during the first 10 days of July. Simultaneously the utilization of soil water ceased. In the greenhouse the growth and utilization of water continued up to the time of maturity. Observations show that the plants in the field matured prematurely,

TABLE 7

The amount of moisture utilized and the transpiration coefficient

	IN TH	E PIELD	IN THE GREENHOUSE			
PLANT	Amount of moisture	Transpiration coefficient	Amount of moisture	Transpiration coefficient		
	poods		poods			
Sunflower	165,313	554	30,311	595		
Belotoorka	140,805	725	7,462	458		
Poltavka	138,465	1,365	12,154	565		
Peas	98,507	524	23,703	655		
Flax	96,757	1,861	24,946	869		
Buckwheat	95,146	232	16,032	200		

were comparatively under-developed, gave a small surface growth, and consequently a very high transpiration coefficient.

In the greenhouse the plants developed normally, gave a normal yield, and yet the transpiration coefficient was very great as compared with other plants. In the transpiration coefficient, as determined by field and greenhouse experiments, flax approaches the plants with a short vegetation period. The large transpiration coefficient of flax under field conditions must be associated with the possibility of the very large evaporation of water indirectly from the soil, since flax does not cover the soil and does not protect it from the sun rays.

UTILIZATION OF WATER IN THE FIELD AND IN THE GREENHOUSE BY VARIOUS PLANTS

By comparing the figures obtained from observations on the utilization of water in the field and in the greenhouse by various plants table 7 was obtained. If the plants were tabulated according to the diminishing value of the trans-

piration coefficient, the following two series would appear: in the field—flax, Poltavka, Belotoorka, sunflower, peas, and buckwheat; in the greenhouse—flax, peas, sunflower, Poltavka, Belotoorka, and buckwheat.

It happens that only the two extreme representatives of the two series coincide; both in the field and in the greenhouse, flax has the greatest transpiration coefficient, and buckwheat, the lowest. As to the places of the other four plants, we see almost a complete contradiction between the field and the greenhouse. The peas and the sunflowers require in the field less water than the wheat; in the greenhouse, on the other hand, wheat requires less water than sunflowers and peas.

An examination of the time of harvest of the plants in the field, shows that the early maturing plants—flax, Poltavka and Belotoorka—have the greatest transpiration coefficient, whereas the late maturing plants have the lowest transpiration coefficient. There are no indications of such a relationship in the greenhouse; here the Belotoorka and Poltavka, which were harvested early, have a lower transpiration coefficient than the plants which matured later, with the exception of buckwheat.

It has been noted already that in the beginning of the growth period the transpiration coefficient for all the field plants is generally very high. This has been ascribed to the fact that there is an indirect evaporation of water from the soil itself, which does not play an essential rôle in the production of the dry matter. That this actually happens is shown by the figures for the determinations of the soil moisture during the growth period of the plants on the cropped plots as compared with the plots that had no vegetation. During the period of sprouting the moisture content of the soil of the cropped and uncropped plots was the same. In either case, the moisture content depended upon the amount of water evaporated indirectly from the soil; upon this, the plant in its first stages of growth had very little effect. The author is convinced that the increase of the transpiration coefficient for the early maturing field plants may be explained by these circumstances. Of course for other plants too the same condition is observed in determining their moisture requirements during the first periods, but for plants with a long vegetation period, soil moisture evaporation does not play a great rôle. Besides, these develop faster than wheat, shade the ground earlier, and prevent the indirect evaporation of soil

If it were possible methodically to exclude the indirect evaporation of water from the soil surface, the transpiration coefficients for the field and greenhouse would approach each other. But this would have theoretical value only. In the field, conditions cannot be changed; a large mass of water must be evaporated indirectly from the soil, which must be taken into consideration in practical wheat culture.

It should be noted that the quick maturing plants—wheat, flax—give in the field and in the greenhouse the least amount of tops as compared with the other plants, which naturally would influence their coefficient. In the field observations the transpiration coefficients for different plants were calculated on the basis of the amount of rainfall between periods of the observations. As has been said, the sum total of this rainfall was not discounted separately, but since it probably influences the moisture content of the soil, it ought to be taken into consideration.

On account of the variations in the growth period of the plants they receive an unequal amount of rainfall, the late maturing plants naturally receiving more than the early maturing. Thus the flax from its sprouting period to the time of harvest receives altogether per desiatina 31,200 poods of rainfall; Poltavka, 36,000; Belatoorka, 50,000; peas and buckwheat, 68,300; and sunflowers, 74,600.

If the total amount of rainfall were taken up by the soil and then utilized for plant development, the amount of such moisture utilized by the different plants for their growth would comprise the following: Poltavka, 26 per cent; flax, 32 per cent; Belatoorka, 36 per cent; sunflowers, 45 per cent; peas, 70 per cent; buckwheat, 72 per cent. The order of plants in this respect is almost identical with that of the transpiration coefficient in the field, with the exception that Poltavka and flax change places.

The remainder of moisture necessary for the plants must have been taken from the soil sources that were present at the time of planting. Undoubtedly a certain portion of the rainfall did not participate in the life processes of the plants: it evaporated indirectly from the soil surface under the influence of the sun rays. This brings out more clearly the importance of the moisture resources of the soil in the spring. It is clear that the quickly maturing plants must have for their successful growth great resources of soil moisture; their successful growth may be taken care of otherwise only by a large amount of rainfall during their growth period. At the same time it becomes clear why cultivated plants with a long vegetation period are successfully grown in this arid district; there is always the chance that a large portion of the required moisture will be covered by the rainfall, which is usually more constant in July and August than in May or June.

WINTER RYE AND WHEAT IN THE FIELD

On account of the difficulty in conducting moisture requirement experiments in the greenhouse, the number of plants studied under such conditions was limited; in the field it was possible to observe a good many plants—winter rye and wheat, corn, sorghum, potatoes, carrots, hay like alfalfa, and others—for which there were no parallels in the greenhouse. The data of only a few of these plants will be considered. To these belong winter rye and wheat on which observations were made from April 20 to July 8 when the winter rye was harvested, and to July 12 when the wheat was harvested. For the winter rye the soil gave up approximately 99,000 poods of water per desiatina. The amount of rainfall per desiatina for this period was 36,000 poods. Thus the winter rye could utilize from the rainfall only 37 per cent of the water necessary

for its growth; it approaches in this respect the earlier spring crops. The transpiration coefficient of winter rye was determined as 545.

The rate of the utilization of water by winter rye was not uniform throughout its growth. Especially large was the utilization of water during the 10-day period from May 23 to June 3—during the time of tube formation to complete heading out when the rye required approximately 36 per cent of the moisture necessary for its growth. During the next 10-day period only 27 per cent of the soil moisture was utilized. During these two 10-day periods the greatest increase in growth of the rye—a total of 86 per cent—was noticeable. Although these figures may not be very accurate, one can judge the rate of accumulation of dry material during crop growth.

For winter wheat the rate of utilization of soil water was very close to that of rye. Of the water necessary for the growth of the winter-wheat—128,500 poods—only 36,000 poods or 28 per cent came in the form of rainfall. The wheat was forced to obtain three-fourths of its moisture from the soil resources present there at springtime. The maximum utilization of soil water by the wheat was the same as for the rye. For the two 10-day periods from June 3 to 23, the winter wheat utilized 58 per cent of all the moisture taken from the soil; after that it utilized relatively very little soil water.

The period of heading out was the most productive in the sense of formation of dry matter of the crop. The final transpiration coefficient for the winter wheat at the time of harvest was 706.

CORN AND SORGHUM IN THE FIELD

Both corn and sorghum are famous for their prolonged period of growth—they were harvested September 5. Since these plants develop very slowly during the first weeks of their growth, the observations were taken in the beginning of June. During this period the soil under corn lost 147,000 poods of water and under the sorghum, 180,800 poods. The rainfall during this time amounted to about 95,000 poods per desiatina. Thus corn could replenish from the rainfall resources as high as 64 per cent of the moisture necessary for its development, and sorghum, about 53 per cent. During the following two 10-day periods from July 3 to 24, corn lost 37 per cent of all the moisture utilized, sorghum lost about 30 per cent. For the corn this period of intensified loss of water coincided with the blooming and the beginning of the filling out of the grain; with the sorghum it coincided with the formation of the head. These periods of intensified utilization of water coincided with the periods of intensified accumulation of dry matter in both plants. During the remaining period of growth, utilization of water was relatively regular.

The final transpiration coefficient for corn is 533; for sorghum, 282. There is reason to believe that the final transpiration coefficient for corn is somewhat too high, since the weight of the dry matter at harvest was found to be lower when taken at earlier periods. Since in securing samples of corn only one or two plants can be taken, errors in picking the plants for sampling are always

possible, and this has its effect on the transpiration coefficient, which in the long run is a result of the division of the total loss of moisture by the weight of the dry matter of the crop. But even in the uncorrected form, the transpiration coefficient of corn and especially that of sorghum is very characteristic.

For potatoes and carrots the greatest amount of growth must be ascribed to the moisture due to rainfall. Thus for potatoes the soil gave up 145,700 poods of water per desiatina, whereas the amount of rainfall during its growth was equal to 129,700 poods, that is, 89 per cent of all the moisture utilized by the potatoes may be attributed to rainfall. For the carrots the figures are somewhat lower; namely, 73 per cent. Utilization of soil water by both of these plants was regular and was not in any way peculiar during a particular 10-day period. The same regularity was observed in the accumulation of the dry matter of the crop.

The final transpiration coefficient for the potatoes was 441; for carrots, 549.

The data of the field observations on the utilization of water by various cultivated plants during 1924 corroborated the fundamental thought expressed on the value of the sources of moisture at the time of planting for the various spring crops and the importance of May rainfall for these plants, and emphasized especially the character of the utilization of water by cultivated plants which are more suited for conditions of rainfall distribution during the vegetation period in that district.

CONCLUSIONS

Under the conditions of the dry year of 1924, the study of the problem of the utilization of water by plants under field and under greenhouse conditions established the fact that the general character of the utilization of water is very similar under the various conditions, but the difficulty of such investigation in the field somewhat obscures the similarity.

1. Under the conditions of this investigation larger amounts of moisture are utilized during the first part of the vegetation period in the field than in the greenhouse. The loss of moisture must be ascribed to the indirect evaporation of water by the soil alongside the plants, something which does not take place in the greenhouse. Therefore, the transpiration coefficients of all the plants in the field are comparatively higher than in the greenhouse during the first part of the vegetation period.

2. This unavoidable loss of water indirectly by the soil during the vegetation period in the field leads to the conclusion that the transpiration coefficient in the field is always greater than in the greenhouse. The isolated cases of a larger transpiration coefficient in the greenhouse then in the field cannot be satisfactorily explained.

3. For the majority of the early spring crops the utilization of water during the growth period is not regular; there are specific periods when the plants put a greater demand on the soil for the moisture. With grain crops this period coincides with the period of heading out and blooming.

4. Utilization of water by seed plants like corn, sorghum and buckwheat, is more regular, but there are certain periods which are outstanding because of greater utilization of moisture.

5. The plants with a long vegetation period—the roots and tubers—are utilizing the soil moisture with a great regularity during the whole period of their growth; no intensified utilization of water at any period was noticed.

6. The relation of the various groups of plants to the soil moisture resources at the time of planting and to the rainfall during the summer is therefore entirely different. Whereas the early maturing grain crops have to depend for their development on the soil water resources, the late maturing plants may utilize the rainfall during the whole vegetation period and thus not be depending so much on the soil resources stored from the spring. The necessity of a thorough preparation of the soil in the fall for planting the early spring crops is very evident from this standpoint. Apparently the fall preparation of the soil will have less effect on the later plants.

7. Notwithstanding the fact that these data cover on'y one year, and that these observations must be continued in order to obtain more substantial conclusions, the author fee's that the resu'ts of the work reported present a certain interest, since the year 1924, which has just

expired, was a typical dry year for the region of the lower Volga.

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